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(54) Title: HIV PROTEASE INHIBITORS

(57) Abstract

Combinatorial libraries of HIV and FIV protease inhibitors are characterized by α-keto amide or hydroxyethylamine core structures flanked by on one side by substituted pyrrolidines, piperidines, or azasugars and on the other side by phenylalanine, tyrosine, or substituted tyrosines. The libraries are synthesized via a one step coupling reaction. Highly efficacious drug candidates are identified by screening the libraries for binding and inhibitory activity against both HIV and FIV protease. Drug candidates displaying clinically useful activity against both HIV and FIV protease are identified as being potentially resistive against a loss of inhibitory activity due to development of resistant strains of HIV.

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HIV PROTEASE INHIBITORS

Specification

Field of Invention:

The invention relates to HIV and FIV protease inhibitors. More particularly, the invention is directed to combinatorial libraries of HIV and FIV protease inhibitors characterized by α-keto amide or hydroxyethylamine core structures flanked by on one side by substituted pyrrolidines, piperidines, or azasugars and on the other side by phenylalanine, tyrosine, or substituted tyrosines. The invention is also directed to methods for making such libraries, to the disclosed compounds made by such method, and to a method for screening such libraries for identifying candidate drugs which have clinically useful activity and which are potentially resistive against loss of inhibitory activity due to development of resistant strains of HIV.

Statement of Government Rights:

This invention was made with government support under National Institutes of Health Grants No.GM 48870 and No. GM 44154 and NCI, DHHS, contract No. N01-CO-46000. The U.S. government has certain rights in the invention.

Background:

Human immunodeficiency virus protease (HIV PR) is an important target for the inhibition of viral replication. Though many potent *in vitro* inhibitors have been developed, most of them are either inactive

or toxic *in vivo*, or mutant forms of the virus emerge which are resistant.(See J.H. Condra, et al., *Nature* (1995): vol. ***374***, 569-571; M. Markowitz, et al., *J. Virol.* (1995): vol. ***69***, 701; D. J. Kempf, et al., *Proc. Natl. Acad. Sci. USA* (1995): vol. *92*, 2484-2488; A.K. Ghosh, et al., *J. Med. Chem.* (1994): vol.*37*, 2506-2508; P.Y. Lam, et al., *Science* (1994): vol. *263*, 380-384; N.A. Roberts, et al., *Science* (1990): vol. *248*, 358; and E.E. Kim, et al., *J. Am. Chem. Soc.* (1995): vol. *117*, 1181-1182.)

The lack of animal systems to test the efficacy of the inhibitors further slows down the drug development process. Recently a similar protease has been identified in the life cycle of feline immunodeficiency virus (FIV). (R.L. Talbott, et al., *Proc. Natl. Acad. Sci. USA* (1989): vol. *86*, 5743-5747; and N.C.Pedersen, et al. *Science* (1987): vol. *235*, 790-793.) FIV is a virus which leads to clinical symptoms comparable to those observed in human acquired immune deficiency syndrome (AIDS). Studies have shown that up to 14% of the cats surveyed in the USA and Canada are infected with FIV. (J.K., Yamamoto, et al., *JAVMA* (1989): vol. 194, 213-220.) In Japan, the figure is 28.9%. (T. Ishida, et al., *JAVMA* (1989): vol. *194*, 221-225.

In drug resistant mutants of HIV, there are at least six cases where HIV PR residues mutate to the structurally aligned residue found in FIV PR. The amino acid changes in HIV PR are V32I (I37-FIV), L90M (M107-FIV), N88D (D105-FIV), I50V (V59'-FIV), K20I

(I25-FIV), and Q29K (K109-FIV). For a list of resistant HIV PR mutants is disclosed by J. W. Mellors, et al. (*International Antiviral News* (1995): vol. 3, 8-13.) The structure alignment was derived from the X-ray crystal structure of FIV PR solved by A. Wlodawer (reference 36). Superimposition of the two proteases based on their X-ray structures indicates similarities between the two proteases and the drug resistant HIV proteases.

HIV PR is a 99 amino acid aspartyl protease which functions as a homodimer. (M.A. Navia, et al., *Nature* (1989): vol. 337, 615-620; and D.D. Loeb, *Virol.* (1989): vol. *63*, 111-121.) FIV PR is also a homodimeric aspartyl protease which consists of 116 amino acid residues. (J.H. Elder, *Infectious Agentsand Disease* (1994): vol. *2*, 361-374.) Both HIV and FIV proteases are responsible for the processing of viral *gag* and *gag-pol* polyproteins into structural proteins and enzymes essential for the proper assembly and maturation of full infectious virions. (S.K. Thompson, *Bioorg. Med. Chem. Lett.* (1994): vol. *4*, 2441-2446.) In particular HIV and FIV proteases show high specificity for the selective cleavage of the Tyrosine/Phenylalanine-Proline amide bonds in the Matrix-Capsid domain of the *gag-pol* polyproteins, a specificity not exhibited by mammalian cellular proteases which are not known to efficiently hydrolyze peptide bonds involving the proline nitrogen. (C. Debouck, *Aids Research and Human Retroviruses* (1992): vol. *8*, 153-164; and J.H. Elder, *J. Virol.* (1993): vol. *67*, 1869-1876.) It is this

specificity that makes HIV PR an attractive target for inhibition. A comparison of the amino acid sequence about the matrix capsid cleavage site (Tyrosine ~ Proline bond) in both HIV and FIV is provided below. As can be seen the residues about the cleavage site are the same at four positions, P_3, P_1, $P_{1'}$ and $P_{2'}$. It is disclosed herein that, due to these similarities, certain HIV PR inhibitors also inhibit FIV PR.

	P_4	P_3	P_2	P_1		$P_{1'}$	$P_{2'}$	$P_{3'}$	P4'
HIVPR	**Ser**	**Gln**	**Asn**	**Tyr**	~	**Pro**	**Ile**	**Val**	**Gln**
FIVPR	**Pro**	**Gln**	**Ala**	**Tyr**	~	**Pro**	**Ile**	**Gln**	**Thr**

Activated ketones, in general, have been shown to inhibit most kinds of proteases (Barrett et. al., *Proteinase Inhibitors;* Research monographs in cell and tissue physiology; *Dingle, J. T., Gordon, J.L., General Eds.; Elsevier Science Publishers;* Amsterdam, 1986). In particular, a recent study shows the design of three different classes of activated ketones which inhibit the aspartyl protease, renin. These potent analogs display IC_{50} values from 4000 nM to 4.1 nM and include 1,1,1-trifluoromethyl ketones, α-keto esters, and α-diketones as the activated ketone functionalities (Patel et. al. *J. Med Chem.* **1993**, *36*, 2431).

The α-keto-amide core structure is isosterically analogous to the activated ketones but is more potent than the reported hydroxyethylamine or phosphinic acid HIV protease inhibitors that are mechanism-based

isosteric core structures.

The α-keto-amide core structure has been used in inhibitors of various enzymes which include serine and cysteine proteases, hydrolases and aminopeptidases. As an example, a series of dipeptidyl and tripeptidyl α-keto amides have been synthesized and evaluated as potent inhibitors for cysteine proteases which include enzymes calpain I, calpain II, cathepsin B, and papain (Li et. al. *J. Med. Chem.* **1993**, *36*, 3472). Another study has identified α-keto amide analogs as inhibitors of an epoxide hydrolase (Wong et. al. *J. Med. Chem.* **1993**, *36*, 211). Additionally, the inhibition of arginyl aminopeptidase (K_i = 1.5 uM), cytosol aminopeptidase (K_i = 1.0 uM) and microsomal aminopeptidase (K_i = 2.5 uM) has been observed from α-keto amide analogs which can be derived from 3-amino-2-oxo-4-phenylbutanoic acid amides. (Rich et. al. *J. Med Chem.* **1992**, *35*, 451).

The activity of dipeptide isosteres is often enhanced by addition of amino acid residues to either the N and C-terminus of the isostere to improve binding in the active site. The prior art provides examples of such inhibitors which include renin, aspartyl proteases and procine pepsin inhibitors (Rich et. al. *J. Med Chem.* **1992**, *35*, 451). The resulting inhibitors generally exhibit high binding affinity to HIV protease. They display, however, instability and/or poor oral bioavailability.

The related art has provided examples of α-keto amide inhibitors of aminopeptidases which contain an unsubstituted proline moiety in the molecule. In particular, Gordon and co-workers have described α-keto amide inhibitors with an unsubstituted proline ring for the themetalloprotease angiotensin converting enzyme (ACE) (Gordon et. al. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 141). Additionally, Arai et. al. (*Chem. Pharm. Bull.* **41**, *9*, 1583) have reported potent inhibitory activity by a prolyl endopeptidase (PEP) inhibitor (N-[N-(4-phenylbutanoyl)-L-prolyl]pyrrolidine).

What is needed are combinatorial libraries of HIV and FIV protease inhibitors and simple synthetic methods for making same.

What is needed is a class of HIV and FIV protease inhibitor having enhanced possibilities of variability at the P_1 and P_1' positions for improving the binding between the enzyme and its inhibitor.

What is needed are methods for screening combinatorial libraries of HIV and FIV protease inhibitors for identifying candidates having both clinically useful inhibitory activity and a potential resistivity to a loss of inhibitory activity due to development of resistant strains of HIV.

What is needed are new HIV and FIV protease inhibitors having clinically useful inhibitory activity and a resistivity to a loss of inhibitory activity due to development of resistant strains of

HIV.

Summary:

It is disclosed herein that FIV PR provides a good model of drug resistance in retroviral proteases. Testing candidate drugs with respect to their inhibitory activity against both HIV and FIV proteases and determining which inhibitors are simultaneously efficacious against both of these mechanistically identical proteases, identifies inhibitors of HIV protease which are potentially less prone to resistance development. Candidate drugs which are successfully screened *in vitro* may then be tested in cats as model systems on which to test HIV PR inhibitors *in vivo*.

Accordingly, one aspect of the invention is directed to a method for identifying a drug candidate as an HIV protease inhibitor potentially resistive against loss of inhibitory activity due to development of resistant strains of HIV. The method employs the following steps:

Step A: determining whether the drug candidate has a binding activity with respect to HIV protease of less than 1 μM;

Step B: determining whether the drug candidate has an inhibitory activity with respect to HIV protease of less than 1 μM;

Step C: determining whether the drug candidate has a binding activity with respect to FIV protease of less than 1 μM; and

Step D: determining whether the drug candidate has

an inhibitory activity with respect to FIV protease of less than 1 µM.

If the drug candidate is determined to have binding and inhibitory activities with respect to both HIV protease and FIV protease of less than 1 µM in each of the above steps, then the drug candidate is selected as an HIV protease inhibitor potentially resistive against loss of inhibitory activity due to development of resistant strains of HIV.

The above method may be applied either to individual drug candidates or to a library of drug candidates.

Another aspect of the invention is directed to methods for synthesizing drug candidates which potentially inhibit HIV protease. More particularly, the drug candidates are of a type which include an N-terminus, a C-terminus, and an α-keto amide core structure linking the N-terminus and the C-terminus. The N-terminus incudes an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrpsines. The aromatic amino acid includes a carbonyl group for linking to and incorporation into the α-keto amide core structure. The C-terminus includes a heterocyclic ring having a ring nitrogen and one or more substitutions. The ring nitrogen of the C-terminus is linked to and incorporated into the α-keto amide core structure. The synthetic method includes the following step:

Step A: providing an N-terminus precursor identical to the N-terminus except that the

carbonyl group is replaced by an α-hydroxyl acid group;

Step B: providing a C-terminus precursor identical to the C-terminus except that the ring nitrogen forms a secondary amine;

Step C: coupling the N-terminus precursor of step A to the C-terminus precursor of step B to form a drug candidate precursor identical to the drug candidate except that the α-keto amide core structure of the drug candidate is replaced by an α-hydroxyl amide core structure linking and incorporating the carbonyl group of the N-terminus and the ring nitrogen of the C-terminus; and then

Step D: oxidizing the α-hydroxyl amide core structure of the drug candidate precursor of step C for forming the α-keto amide core structure and the drug candidate.

The above synthetic method may also be adapted for synthesizing combinatorial libraries of HIV and FIV protease inhibitors containing nxm drug candidates. The above synthetic method is modified by providing nxm reaction vessels, loading each of the **n** N-terminus precursors into **m** of the reaction vessels, and then loading each of the **m** C-terminus precursors into **n** of reaction vessels so as to form nxm admixtures of N-terminus precursor and C-terminus precursors. Then, each of the nxm admixtures is allowed to undergo a coupling reaction in which the N-terminus precursor couples to the C-terminus precursor to form nxm drug candidate precursors. The drug precursor candidates are identical to the nxm

drug candidates except that the α-keto amide core structure of the nxm drug candidates is replaced by an α-hydroxyl amide core structure linking and incorporating the carbonyl group of the N-terminus and the ring nitrogen of the C-terminus. Finally, the α-hydroxyl amide core structure of each of the nxm drug candidate precursors is oxidized so as to form the α-keto amide core structure of the desired drug candidate.

A further aspect of the invention is directed to combinatorial libraries nxm drug candidates characterized by having a α-keto amide core structure and a potential inhibitory activity with respect to HIV protease. The drug candidates are characterized by having an N-terminus selected from **n** N-termini where **n** is two or greater, a C-terminus selected from **m** C-termini where **m** is two or greater, and a α-keto amide core structure which links the N-terminus to the C-terminus. Each of the **n** N-termini incudes an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrosine. The aromatic amino acid includes a hydroxyethyl group *in lieu* of a carbonyl group which is linked to and incorporated into the α-keto amide core structure. Each of the **m** C-termini includes a heterocyclic ring having a ring nitrogen and one or more substitutions. The ring nitrogen of the C-terminus is linked to and is incorporated into the α-keto amide core structure.

Another aspect of the invention is directed to combinatorial libraries of nxm drug candidates

characterized by having a hydroxyethylamine core structure and a potential inhibitory activity with respect to HIV protease. The drug candidates are characterized by having an N-terminus selected from **n** N-termini where **n** is two or greater, a C-terminus selected from **m** C-termini where **m** is two or greater, and a hydroxyethylamine core structure which links the N-terminus to the C-terminus. Each of the **n** N-termini incudes an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrosine. The aromatic amino acid includes a hydroxyethyl group *in lieu* of a carbonyl group which is linked to and incorporated into the hydroxyethylamine core structure. Each of the **m** C-termini includes a heterocyclic ring having a ring nitrogen and one or more substitutions. The ring nitrogen of the C-terminus is linked to and is incorporated into the hydroxyethylamine core structure.

Another aspect of the invention is directed to a series of improved mechanism based inhibitor of HIV or FIV aspartyl protease. Each of the improved mechanism based inhibitors is of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus. The N-terminus incudes an aromatic amino acid residue linked to the core structure. The C-terminus includes a heterocyclic ring having a ring nitrogen linked to the core structure. The core structure is isosteric with a scissile amide bond of an HIV or FIV aspartyl protease substrate.

In the first embodiment of improved mechanism based inhibitor, the core structure is an α-keto amide and the heterocyclic ring of the N-terminus is a pyrrolidine having at least one substituant other than carboxylic acid and carboxymethyl ester. Examples of this first embodiment of improved mechanism based inhibitors are provided as follows:

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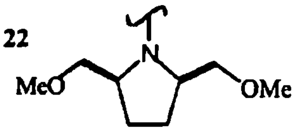

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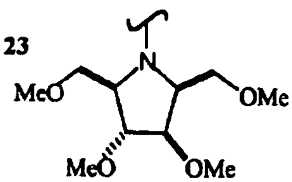

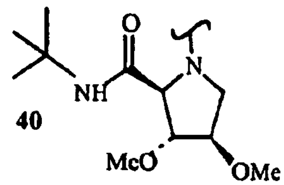

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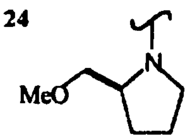

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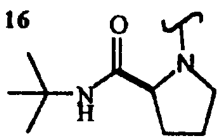

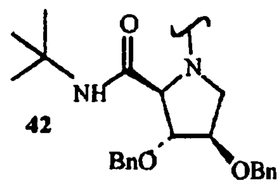

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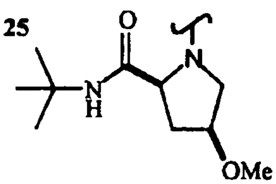

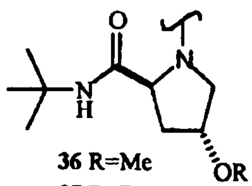

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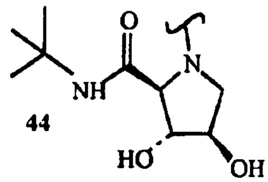
37 R=Bn



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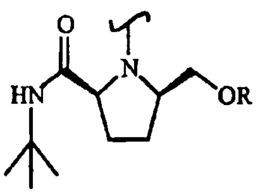
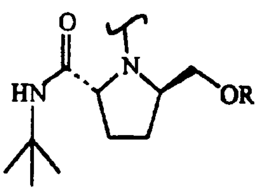


46 R=H; 47 R=Bn, 48 R=Me



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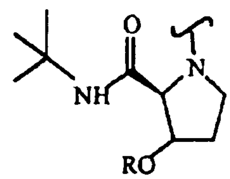
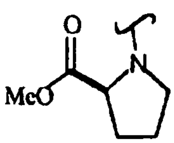


58 R=H; 59 R=Bn, 60 R=Me (cis
64 R=H; 65 R= Bn, 66 R=Me (tr



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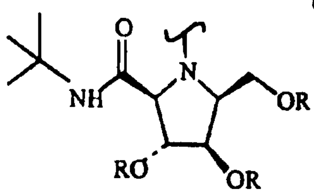
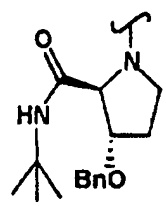


101 R=H; 102 R=Bn, 103 R=Me



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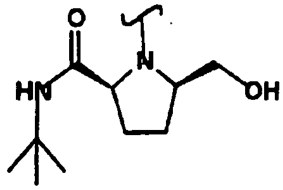

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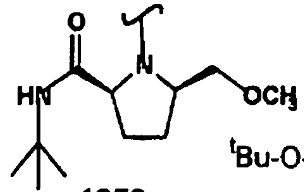

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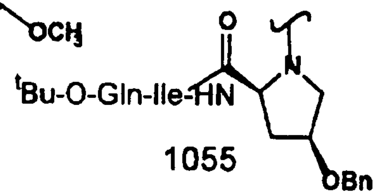

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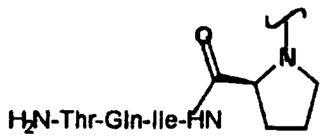

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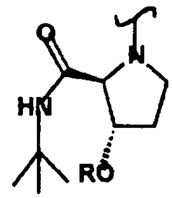

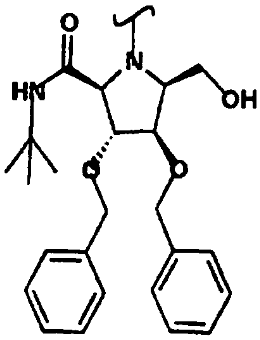

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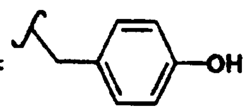
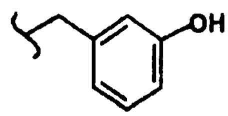
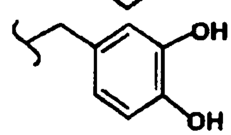
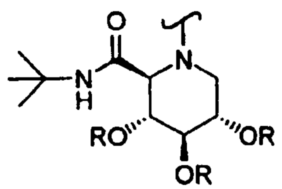
1054 R = 

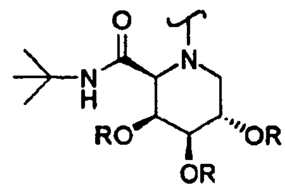
1059 R = 

1060 R = 

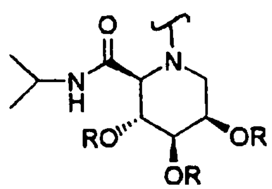
In a second embodiment of improved mechanism based inhibitor, the core structure is an α-keto amide and the heterocyclic ring of the N-terminus is a piperadine or azasugar. Examples of piperadines and azasugars employed in this second embodiment of improved mechanism based inhibitors are provided as follows:



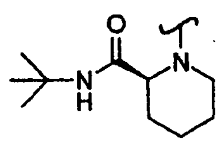
R = 85 H, 86 Me, 87 Bn



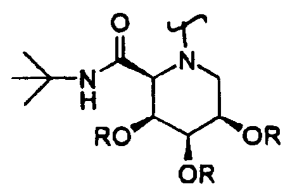
R = 88 H, 89 Me, 90 Bn



R = 91 H, 92 Me, 93 Bn

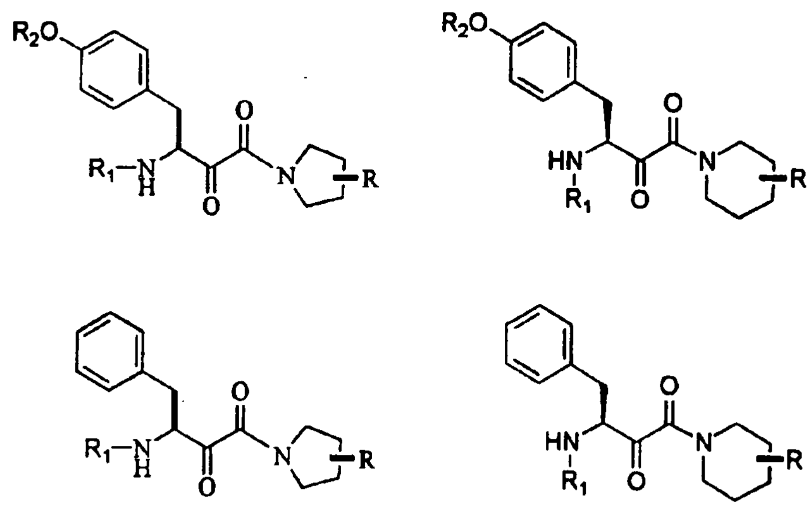


94



R = 95 H, 96 Me, 97 Bn

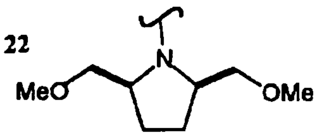
In a third embodiment of improved mechanism based inhibitor, the core structure is an α-keto amide and the aromatic amino acid of the C-terminus is selected from a group consisting of tyrosine having a protected amino, tyrosine having a protected amino and a substituted hydroxyl, and phenylalanine having a protected amino protected by carbobenzyloxy. Examples of this third embodiment of improved mechanism based inhibitors are provided as follows:



wherein R is selected from the group consisting of hydrogen, hydroxy, benzyloxy, alkyl$_{(C1-C4)}$-oxy, *o*-methoxy-benzyloxy, *m*-methoxy-benzyloxy, *p*-methoxy-benzyloxy, *o*-methoxy-nitrobenzyloxy, *m*-methoxy-nitrobenzyloxy, *p*-methoxy-nitrobenzyloxy, acetonide, benzylidene, 3-oxymethyl-catechol, 4-oxymethyl-catechol; R_1 is selected from the group consisting of carbobenzyloxy (CBZ), *tert*-butoxycarbonyl (*t*-BOC), acyl; R_2 is selected from the group consisting of hydrogen, benzyl, alkyl$_{(C1-C4)}$, *o*-methoxy-benzyl, *m*-methoxy-benzyl, *p*-methoxy-benzyl, *o*-methoxy-nitrobenzyl, *m*-methoxy-nitrobenzyl, *p*-methoxy-nitrobenzyl, 3-methylene-catechol, 4-methylene-catechol.

In a fourth embodiment of improved mechanism based inhibitor, the core structure is an

hydroxyethylamine and the heterocyclic ring of the N-terminus is a pyrrolidine having at least one substituant other than carboxylic acid and carboxymethyl ester. Examples of pyrrolidines employed in this fourth embodiment of improved mechanism based inhibitors are provided as follows:

22


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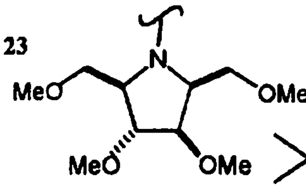

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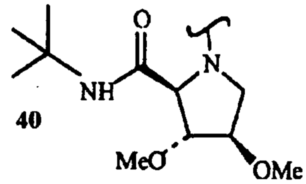

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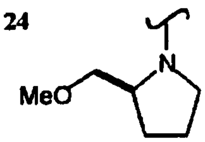

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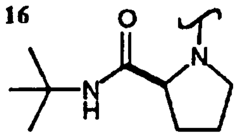

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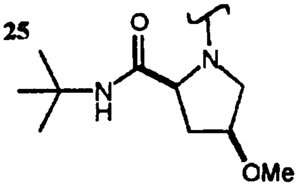
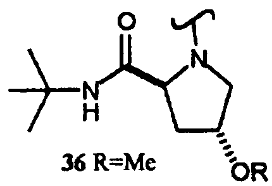
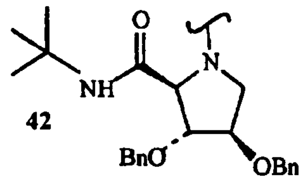
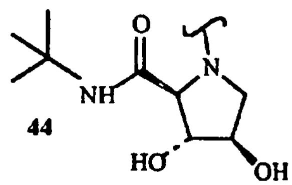
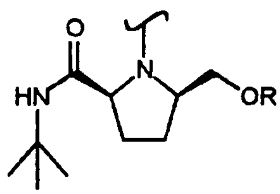
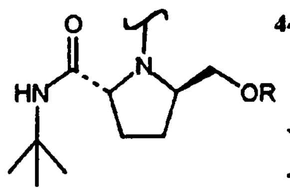


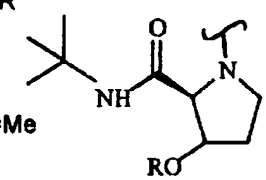

36 R=Me
37 R=Bn

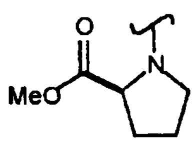
42


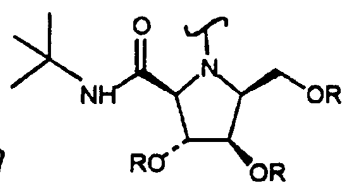
44


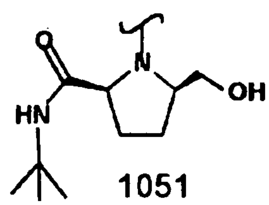

46 R=H; 47 R=Bn, 48 R=Me


52 R=H; 53 R=Bn, 54 R=Me

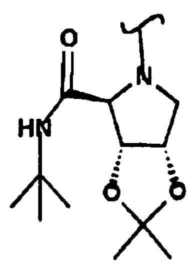

58 R=H; 59 R=Bn, 60 R=Me (cis)
64 R=H; 65 R= Bn, 66 R=Me (tra


100

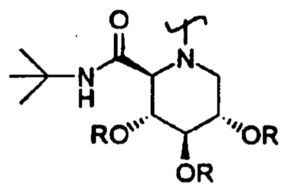

101 R=H; 102 R=Bn, 103 R=Me


1051

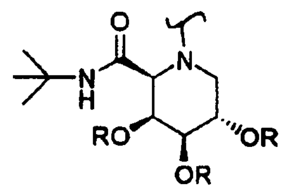
and


1058

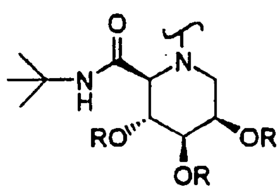
In a fifth embodiment of improved mechanism based inhibitor, the core structure is hydroxyethylamine and the heterocyclic ring of the N-terminus is a piperadine or azasugar. Examples of piperadines and azasugars employed in this fifth embodiment of improved mechanism based inhibitors are provided as follows:



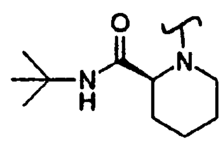
R = 85 H, 86 Me, 87 Bn



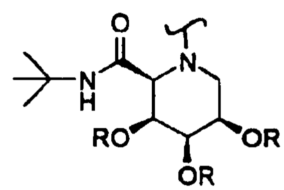
R = 88 H, 89 Me, 90 Bn



R = 91 H, 92 Me, 93 Bn

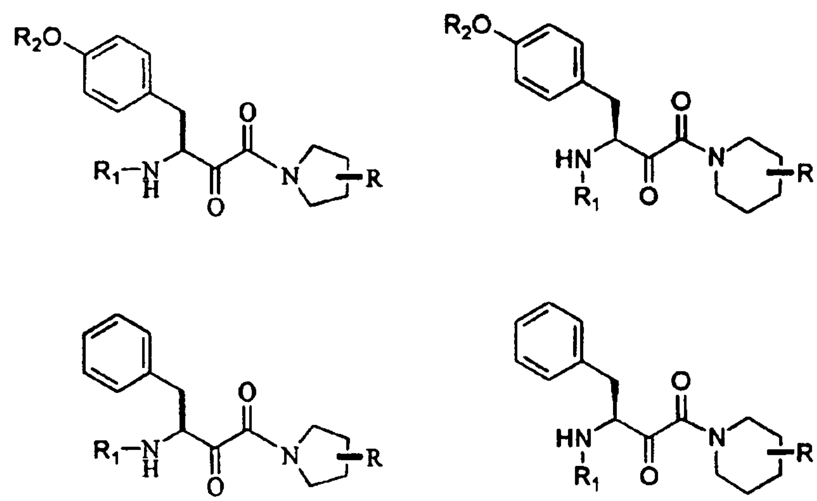


94



R = 95 H, 96 Me, 97 Bn

In a sixth embodiment of improved mechanism based inhibitor, the core structure is hydroxyethylamine and the aromatic amino acid of the C-terminus is selected from a group consisting of tyrosine having a protected amino, tyrosine having a protected amino and a substituted hydroxyl, and phenylalanine having a protected amino protected by carbobenzyloxy. Examples of this sixth embodiment of improved mechanism based inhibitors are provided as follows:



wherein R is selected from the group consisting of hydrogen, hydroxy, benzyloxy, alkyl$_{(C1-C4)}$-oxy, *o*-methoxy-benzyloxy, *m*-methoxy-benzyloxy, *p*-methoxy-benzyloxy, *o*-methoxy-nitrobenzyloxy, *m*-methoxy-nitrobenzyloxy, *p*-methoxy-nitrobenzyloxy, acetonide,

benzylidene, 3-oxymethyl-catechol, 4-oxymethyl-catechol; R_1 is selected from the group consisting of carbobenzyloxy (CBZ), *tert*-butoxycarbonyl (*t*-BOC), acyl; R_2 is selected from the group consisting of hydrogen, benzyl, alkyl$_{(C1-C4)}$, *o*-methoxy-benzyl, *m*-methoxy-benzyl, *p*-methoxy-benzyl, *o*-methoxy-nitrobenzyl, *m*-methoxy-nitrobenzyl, *p*-methoxy-nitrobenzyl, 3-methylene-catechol, 4-methylene-catechol.

Description of Figures

Figure 1 illustrates an approach to rapidly access a number of potential inhibitors of HIV and/or FIV proteases to determine the protecting group and ideal substitution pattern of the 'proline' moiety to provide maximum inhibition of the enzymes.

Figure 2 illustrates a comparison of the α keto amide core structure to other isosteric structures. Compound activities are indicated.

Figure 3 illustrates hydrogen bond interactions between a hydroxyethylamine isostere and the active site of HIV PR as observed from X-ray crystallography.

Figure 4 illustrates a general acid - base mechanism for inhibitor **2** interaction with HIV PR aspartate groups.

Figure 5 illustrates inhibitory activity of variously substituted pyrrolidine analogues against

HIV PR.

Figure 6 illustrates the synthesis of compound **11.** The steps indicated are as follows: (i) *t*-BuLi, ethyl vinyl ether, $MgBr_2$, THF; (ii) O_3, CH_2Cl_2; (iii) 0.17N LiOH, $MeOH/H_2O$, 2:1, 98%.

Figure 7 illustrates the synthesis of compound **2.** The steps indicated are as follows: (i) $NaBH_4$, MeOH, 0 °C, 95%; (ii) 0.17 N LiOH, $MeOH/H_2O$, 2:1, 98%; (iii) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1), 84%; (iv) Dess-Martin periodinane, CH_2Cl_2, 95%.

Figure 8 illustrates the synthesis of compound **3.** The steps are indicated as follows: (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1), 80%; (ii) Dess-Martin periodinane, CH_2Cl_2, quantitative.

Figure 9 illustrates the synthesis of compounds **31** and **32** with the indicated substrate and reagents.

Figure 10 illustrates the synthesis of compound **35** with the indicated substrate and reagents.

Figure 11 illustrates a general coupling procedure to form hydroxyethyl amine compounds starting from epoxide **21** and using the indicated pyrolidines. The procedure is as follows: (i) Methanol, Et_3N, reflux, 24 hours, 40-60%.

Figure 12 illustrates a general coupling procedure to form α-ketoamide compounds starting from

carboxyacid **15** and using the indicated pyrolidines. The two step procedure is as follows: (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1); (ii) Dess-Martin periodinane oxidation, CH_2Cl_2, quantitative.

Figure 13 illustrates a general coupling procedure to form α-ketoamide compounds starting from carboxyacid **15** and using the indicated substituted piperidines. The two step procedure is as follows: (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1); (ii) Dess-Martin periodinane oxidation, CH_2Cl_2, quantitative.

Figure 14 illustrates a general coupling procedure to form hydroxyethyl amine compounds starting from epoxide **21** and using the indicated substituted piperidines. The procedure is as follows: (i) Methanol, Et_3N, reflux, 24 hours, 40-60%.

Figure 15 illustrates the synthesis of pyrolidines **46**, **47** and **48** with the indicated substrate and reagents.

Figure 16 illustrates the synthesis of pyrolidines **52**, **53** and **54** with the indicated substrate and reagents.

Figure 17 illustrates the synthesis of pyrolidine **44** with the indicated substrate and reagents.

Figure 18 illustrates the synthesis of

pyrolidine **40** with the following steps: (i) CBZ-Cl, methylene chloride; (ii) TBDPSCl, Et_3N, DMF; (iii) Methyl iodide, sodium hydride, DMF; (iv) TBAF, THF; (v) 1.5 M H_2SO_4, CrO_3, acetone; (vi) H_2N^tBu, EDC, methylene chloride; (vii) H_2, $Pd(OH)_2$ on Carbon, MeOH.

Figure 19 illustrates the synthesis of pyrolidine **42** with the following steps: (i) BOC-ON, methylene chloride; (ii) TBDPSCl, Et_3N, DMF; (iii) Benzyl bromide, sodium hydride, DMF; (iv) TBAF, THF; (v) 1.5 M H_2SO_4, CrO_3, acetone; (vi) H_2N^tBu, EDC, methylene chloride; (vii) H_2, $Pd(OH)_2$ on Carbon, MeOH.

Figure 20 illustrates the synthesis of pyrolidines **58**, **59**, **60**, **64**, **65**, and **66** with the following steps: For compounds **58, 59, 60:** (i) CBZ-Cl, methylene chloride; (ii) TBDMSCl, Et_3N, DMF; (iii) H_2N^tBu, EDC, methylene chloride; (iv) TBAF, THF; (v) "Mitsunobu" procedure: PPh_3, DEAD (diethylazaodicarboxylate), $ClCH_2CO_2H$, then water, pyridine to pH 6.7; (vi) Benzyl bromide for **59** or methyl iodide for **60**, sodium hydride, DMF (omit this step for **58**) (vii) H_2, $Pd(OH)_2$ on Carbon, MeOH. For compounds **64**, **65**, and **66** same conditions as above without step (v) "Mitsunobu" procedure.

Figure 21 illustrates the synthesis of piperidines **86 and 87** with the following steps: (i) CBZ-Cl or BOC-ON, methylene chloride; (ii) TBDPSCl, Et_3N, DMF; (iii) Benzyl bromide for **87** or methyl iodide for **86**, sodium hydride, DMF (iv) TBAF, THF; (v) 1.5 M H_2SO_4, CrO_3, acetone; (vi) H_2N^tBu, EDC, methylene chloride; (vii) H_2, $Pd(OH)_2$ on Carbon, MeOH.

Figure 22 illustrates the synthesis of piperidines **85** with the following steps: (i) CBZ-Cl or BOC-ON, methylene chloride; TBDPSCl, Et_3N, DMF; (ii) acetic anhydride, triethylamine, DMF (iii) TBAF, THF; (iv) 1.5 M H_2SO_4, CrO_3, acetone; (v) H_2N^tBu, EDC, methylene chloride; (vi) NaOMe, MeOH; (vii) H_2, $Pd(OH)_2$ on Carbon, MeOH.

Figure 23 illustrates tyrosine derivatives of pyrolidine or piperidine containing α-ketoamide and hydroxylethylamine core structures to probe for FIV and HIV protease selectivity.

Figure 24 illustrates targeted variable sites on the core structure which can be assessed using a combinatorial approach. Di-/tri-/tetrameric substrates for HIV-PR and FIV-PR are possible. Candidate molecules display activity by cleavage of either HIV-PR or FIV-PR protease. The candidate molecules are subsequently rederivatized via replacement of the scissile amide bond to an α-keto amide or hydroxyethyl amine, affording further potential inhibitors. The synthetic strategy involves solution phase coupling of the added residues (standard coupling conditions, EDC HOBT, CH_2Cl_2 or DMF) to afford di-/tri-/tetra peptides. Simple acidic and basic washes afford the desired peptides cleanly, with no purification necessary. Compounds are then dissolved in a minimal amount of DMSO and added to buffer solution, arranged in 96-well micro-titer plates. Addition of the enzyme and subsequent mass spectral analysis identifies the cleaved substrates as hits. These molecules are then

rederivatized with an α-keto amide or hydroxyethyl amine core unit and tested for inhibitory activity.

Figure 25 illustrates the creation of non-linear protease inhibitors using the up and down search for new complementary binding sites attached to a designed mechanism-based core structure.

Figure 26 illustrates various α-ketoamide and hydroxyethyl HIV protease inhibitors.

Figure 27 illustrates the synthesis of α-ketoamide compound **1050** with the indicated substrate and reagents.

Figure 28 illustrates the synthesis of hydroxyethylamine compound **1051** with the indicated substrate and reagents.

Figure 29 illustrates the synthesis of α-ketoamide compound **1053** with the indicated substrate and reagents.

Figure 30 illustrates the synthesis of α-ketoamide compounds **1054, 1059, and 1060** with the indicated substrates and reagents.

Figure 31 illustrates the synthesis of α-ketoamide compound **1055** with the indicated substrate and reagents.

Figure 32 illustrates the synthesis of α-ketoamide compound **1056** with the indicated substrate and reagents.

Figure 33 illustrates the synthesis of α-ketoamide compound **1057** with the indicated substrate and reagents.

Figure 34 illustrates the synthesis of α-ketoamide compound **1058** with the indicated substrate and reagents.

Detailed Description:

One aspect of the invention is directed to a new class of the HIV/FIV PR inhibitors, viz. α-keto amide inhibitors. The mode of action of the α-keto amide inhibitor is of particular interest as it represents a new type of mechanism-based enzyme inhibition which can lead to the development of tight-binding inhibitors to overcome the problem of resistance.

When assayed against HIV PR, the novel α-keto amide **1** (Figure 3) was found to have a K_i of 6 μM. Subsequent studies have shown that a simple modification of the N- and C-terminal protecting groups to give **2** (Figure 3) enhances the potency of this core isostere against HIV PR, to give a K_i of 214 nM.

The increase in activity in compound **2** may be due to favorable hydrophobic interactions between the protecting groups (i.e. the Cbz-protecting group and the *t*-Bu group) and the active site of HIV PR. The Boc-protecting group is also hydrophobic but is shorter and sterically more bulky, making it unable to extend effectively into the appropriate hydrophobic binding pocket. This result demonstrates how simple modifications of the core isostere can significantly improve its potency. It is disclosed that the potency of the α-keto amide **2** can be improved by introduction of additional complementary groups to the proline ring moiety. Computer modeling (Insight/Discover) indicates that attachment of hydrophobic groups to the proline ring moiety will

enhance binding. It is disclosed that addition of a *cis*-benzyl ether to C-4 of the proline moiety increased binding 3-fold (K_i = 65nM).

The activity of the isostere **2** can be then compared to that of the identically substituted α-hydroxy amide precursors **23** and **24**. The *S*-diastereomer **24** (IC_{50} = 2μM) is disclosed to be more potent than the *R*-diastereomer **23** (IC_{50} = 300 μM), but less active than **2**. The high potency of the α-hydroxy amide **24** implies that the hydroxyl group is hydrogen bonding more effectively with the catalytic carboxylic acid groups of HIV PR than in compound **23**, similar to that observed in the X-ray structure of a hydroxyethylamine inhibitor enzyme complex (Figure 4). The stereochemistry of the isosteres **23** and **24** was determined by ^{1}H NMR studies on the *R*- and *S*-Mosher esters derived from the *S*-α-hydroxy ester **19**.

The ketone moiety of the α-keto amide **2** is disclosed to be hydrated and is hydrogen bonding in a similar manner to that of compound **23**. However in this case, ^{13}C NMR studies on compound **2** in deuterated DMSO/D_2O (5:1) indicates that, in the presence of water, the ketone moiety of the α-keto amide *remains unhydrated* even after incubation for 24 hours (Figure 5). This indicates that the ketone moiety of **2** is stable in the presence of water and is therefore difficult to hydrate in the absence of a catalyst. It is likely that hydration of the ketone moiety takes place within the active site of HIV PR as

illustrated in Figure 6, and the resulting hydrate is then stabilized through hydrogen bonding interactions with the aspartate residues of the enzyme. The hydrated form of **2** is considered to be a good transition state mimic based on the model presented in Figure 6.

Time dependent assays do not exhibit time dependent inhibition, indicating that if the active form of the α-keto amide **2** is indeed the hydrate, the hydration step must be rapid or **2** itself is the active form. To further investigate the mode of action, the X-ray structure of the complex between the α-keto amide **2** and HIV PR was determined and the result indicated that the α-keto amide is hydrated, supporting the enzyme-assisted hydration mechanism (Figure 7).

As seen in the electron density map, the inhibitor is bound in the active site of HIV-PR in its hydrated state (Figure 7). One of the two hydroxyls is located between two aspartates, making hydrogen bonds with both of them, while the other hydroxyl interacts with only one aspartate. The keto group is also hydrogen bonded to a catalytic aspartate. The phenylalanine and proline side chains occupy proper S_1 and S_1' pockets making interactions similar to those observed in other structures. The carboxybenzyl group on the N-terminus and the tert-butyl group of the tert-butyl amide on the C-terminus of the inhibitor are roughly in the S_2 and S_2'pockets, respectively. The conserved water (Wat-301), which

is forming hydrogen bonds between the inhibitor and the two flaps of the enzyme, is clearly observed. Its location is rather asymmetric, unlike the case of the majority of other inhibitor complexes. The distance between the carbonyl oxygen which mimics the P_2 CO group and Wat-301 is 2.5 Å, while the distance to the P_1'CO is 3.2 Å.

Remarkable similarity is observed in the binding mode of the central and C-terminal parts of compound 2, and the similar parts of an inhibitor KNI-272. The differences in their binding constants are very significant (K_i for KNI-272 is almost five orders of magnitude lower than for **2**), despite the presence of an extra hydrogen bond in the central part of the complex of HIV PR and compound **2**. Thus the increase in the potency of KNI-272 must be due to the elongation of its N-terminus, which has a bulky 5-isoquinolyloxyacetyl (IQoa) moiety. The regions of the protein interacting with this group, including the Phe-53 in one monomer and Pro-81 in another shift significantly towards the inhibitor, making extensive hydrophobic contacts with the IQoa group.

When the α-keto amide **2** was tested against FIV PR, it was found to have no inhibitory effect when added in concentrations up to 70 mM. This result was surprising due to the similarity between the natural substrates for HIV and FIV proteases bordering the matrix/capsid cleavage site as illustrated earlier. It appears that FIV PR may require additional specific residues between the P_4 - P_4'sites, than HIV PR, before it is able to recognize the core isostere

2 as a substrate. This is also supported by the observation that HIV PR will cleave an Acetyl-(6 residue) peptide substrate of sequence Gln-Ala-Tyr~Pro-Ile-Gln whereas the smallest peptide FIV PR is known to cleave is an acetyl-(8 residue) peptide of sequence Pro-Gln-Ala-Tyr~Pro-Ile-Gln-Thr. The corresponding protected dipeptide (Cbz-Phe-Pro-NBu^t) was not cleaved by HIV or FIV proteases under normal assay conditions.

In an effort to develop inhibitors of the FIV PR, it was found that the addition of suitable residues to interact with just the P_2'and P_3' sites of FIV PR was sufficient for moderate inhibition. Coupling of a side chain specific for FIV PR to the C-terminus of **2** gave **4** (Figure 8). This extended isostere **4** was found to have an IC_{50} of 25μM and a *K*i of 29 μM against FIV PR and the activity against HIV PR was slightly enhanced (K_i of 154 nM). It appears that the isosteric core structure of HIV PR inhibitors do not bind tightly to the FIV PR, and additional complementary groups are needed to enhance the binding. This difference is also observed in the analysis of other known HIV PR inhibitors. The potent cyclic urea based HIV PR inhibitor DMP 323 (IC_{50} = 36nM, K_i = 0.27nM) for example was also found to be a very poor inhibitor of FIV PR (IC_{50} = 7.3mM).

When the activity of the monomethylated derivative **7** is compared to that of the dimethylated derivative **5**, it can be seen that a hydrophobic moiety at the C-5 position decreases the potency of the isostere, whereas the activity increases when

similar hydrophobic substitutions are made at the C-3 and C-4 positions as in compound **6**. The permethylated pyrrolidine derivative **6** was the most potent derivative of the series **5-7**, but significant activity was lost due to the absence of the amide bond at C-1, as can be seen by comparison of the activity of the methylated derivatives **5-7** to that of compound **8**. The C-4 substituted pyrrolidine derivatives **9-11** were shown to be more potent than **8**, with the *trans*-C-4 methoxy derivative **10** being the best (IC_{50} = 2.9 μM).

The permethylated α-keto amide derivative **12** (Figure 10) was synthesized for comparison and was found to be more potent than the corresponding hydroxyethylamine isostere **6** but significantly less potent (K_i = 20µM) than the original isostere **2** against HIV PR. This result again illustrates the importance of an amide bond at C-1 of the pyrrolidine derivative.

In summary, a new class of mechanism based inhibitors of the HIV and FIV proteases with pyrrolidine-containing α-keto amide and hydroxyethylamine core structures is disclosed. These α-keto amide core structure **2** are disclosed to be approximately 300-fold better than the corresponding hydroxyethylamine isosteric structure and 1300-fold better than the corresponding phosphinic acid derivative as an inhibitor of the HIV protease. The α-keto amide is however not hydrated until it is bound to the HIV protease as indicated by

the NMR study and the X-ray structural analysis. Further analysis of the inhibition activities of hydroxyethylamine isosteres containing modified pyrrolidine derivatives disclosed that a *cis*-methoxy group at C-4 of the pyrrolidine improved the binding 5-fold and 25-fold for the *trans*-isomer. When this strategy was applied to the α-keto amide isostere, a *cis*-benzyl ether at C-4 was found to enhance binding 3-fold. Of the core structures prepared as inhibitors of the HIV protease, none show significant inhibitory activity against the mechanistically identical FIV protease, and additional complementary groups are needed to improve inhibition.

Another aspect of the invention is directed to the use of FIV PR as a model for the development of HIV PR inhibitors and for the study of drug resistance. This disclosure is supported by the data reported herein and by several additional observations. First, both enzymes are mechanistically identical. Second, structure-based alignment of the two enzymes displays their structure similarity (Figures 11 and 12). Third, most potent non-covalent inhibitors of the HIV PR are not good inhibitors of the FIV PR, whereas FIV PR inhibitors are often better HIV PR inhibitors. Therefore, good low molecular weight inhibitors of the FIV PR are disclosed to be better for the HIV PR. Fourth, the HIV PR isolated from the inhibitor-resistant mutants contains mutations that are found in the corresponding positions of the FIV PR. As shown in Figure 12, the six highlights represent the changed amino acids found in the HIV PR isolated from mutants

resistant to HIV PR inhibitors which are identical to those found in the FIV PR in the same position. Accordingly, FIV PR is disclosed to be a good model for the development of HIV PR inhibitors with less resistance problem. For example, the HIV PR isolated from a mutant resistant to a synthetic protease inhibitor contains the I50V mutation, and the mutant protease is less inhibited by the same inhibitor by 80-fold.

Table I. Inhibition activities against HIV and/or FIV PR. The inhibitory activities of various isosteres against the HIV and/or FIV PR illustrating a dual screening method against these related viral proteases are illustrated in Table I provided below:

Table I. Inhibition activities against HIV and/or FIV PR.

	HIV PR		FIV PR	
Compound	IC_{50}	K_i	IC_{50}	K_i
1	6μM	6μM	-	-
2	700nM	214nM	>70mM	-
2a	1.57μM	220nM		
2b	1.9μM	318nM		
2c	460nM	65nM		
3	3.18μM	405nM	-	-
4	-	154nM	25μM	29μM
5	1.8mM	-	-	-
6	100μM	-	-	-
7	1.6mM	-	-	-
8	60μM	-	-	-
9	17μM	-	-	-
10	2.9μM	-	-	-
11	3.9μM	-	-	-
12	20μM	-	-	-

Synthetic Methods

1) Biological Assays

For determination of IC_{50} values for HIV protease, a backbone engineered HIV-1 protease, prepared by total chemical synthesis (Kent et. al. Science 1992, 256, 221) 450 nM final concentration was added to a solution (152 µL final volume) containing inhibitor, 28 µM fluorogenic peptide substrate (sequence Abz-Thr-Ile-Nle-Phe-(p-NO_2)-Gln-Arg-NH_2 (Toth et. al., Int. J. Peptide Res. 1990, 36, 544)) and 1.8 % dimethylsulfoxide in assay buffer: 100mM MES buffer containing 0.5 mg/mL BSA (Bovine Serum Album, fatty acid, nuclease and protease free - to stabilize enzyme) at pH 5.5. The solution was mixed and incubated over 5 minutes during which time the rate of substrate cleavage was monitored by continuously recording the change in fluorescence of the assay solution. An excitation filter of 325 nm, and an emission filter of 420 nm were used. This data was converted into µM substrate cleaved per minute, using a predetermined standard calibration curve of change in fluorescence against concentration of substrate cleaved.

Determination of K_i for HIV protease was performed similarly with the following modifications. The substrate concentrations used were 57, 43, 28 and 14 µM. All other concentrations were as above. The curve fit for the data was determined and the subsequent K_i derived using a computer program based on the equation of Morrison et. al. BioChim. Biophys. ACTA 1969,185, 269, for tight binding

inhibitors.

For determination of K_i and IC_{50} for FIV protease, 0.125 µg of the enzyme was added to a solution (100µL final volume) containing inhibitor, 560 µM peptide substrate (sequence Gly-Lys-Glu-Glu-Gly-Pro-Pro-Gln-Ala-Tyr~Pro-Ile-Gln-Thr-Val-Asn-Gly) and 2% dimethyl sulfoxide in a 1:3 mixture of assay buffer (as above) and 4M $NaCl_{aq.}$ solution. The solution was mixed and incubated for 10 minutes at 37 °C and the reaction quenched by addition of 8M guanidine HCl solution containing 0.2 M sodium acetate at pH 4.2 (100 µL). The cleavage products and substrate were separated by reverse phase HPLC. Absorbance was measured at 215 nm, peak areas were determined and percent conversion to product was calculated using relative peak areas. The data were plotted as 1/V (V = rate substrate is cleaved in nmol/min) against inhibitor concentration and the $-K_i$ determined as the point at which the resulting line intersects with $1/V_{max}$ (V_{max} = 6.85 nmol/min). IC_{50} was determined as the inhibitor concentration at 50% inhibition. V_{max} (6.85 ± 0.7 nmol min^{-1}) and K_m (707 ±70µM) for FIV protease were determined from a plot of 1/V (V = rate in nmol/min) against 1/[S] ([S] = substrate concentration in nmol). The data used was generated similarly to that for K_i with the following modifications. The substrate concentrations used were 560, 448, 336, 224, 111 and 56 µM, in the absence of inhibitor.

Purification of FIV protease: A 503 base pair Eco R1-Bam H1 fragment containing the coding sequence of FIV protease was cloned from FIV-34TF10 (Talbott et. al. Proc. Natl. Acad. Sci. USA 86 1989, 5743)

into the pT7-7 vector (Tabor et. al. Proc. Natl. Acad. Sci USA 82 1985, 1074). The 5' end of the insert was modified by the addition of an Ndel adaptor, which provided the proper reading frame with initiation of translation from the methionine encoded in the latter site. Translation resulted in production of an 18.6 kDa precursor, which auto-processed to a 13.2 kDa FIV PR plus N- and C-terminal fragments of 3.6 kDa and 1.8 kDa, respectively. The construct was transformed into *E. coli* strain BL21.DE3, lys S (Studier et. al. Meth. Enzymol. 1990, 185, 60) and overnight cultures were used to inoculate 15 liter fermentations, performed using Circlegrow medium (Bio 101) plus 100 µL ampicillin, 20 µM chloramphenicol, at 37 °C. The cells were allowed to reach mid-log phase, then the temperature was reduced to 24 °C and IPTG (isopropylß-thiogalactopyranoside) was added to a final concentration of 1 mM. The fermentation was allowed to proceed for 16 hours, at which time the cells were harvested by centrifugation and frozen at -70 °C in 100 g aliquots for future use.

Cells (100 g) were lysed by addition of 600 mL, 50 mM Tris-HCl, pH 8, 5 mM EDTA and 2 mM 2-mercaptoethanol to the frozen pellet. The cells lysed upon thawing and the viscous mixture was homogenized at 4 °C for 2 min in a Waring blender. The sample was centrifuged at 8,000 x g for 20 min and the pellet discarded. The sample was diluted to 1 liter, then subjected to tangential flow against a 300 K cut-off membrane (Filtron) and the PR was washed through the membrane using five liters of the same buffer. The retentate was discarded and the

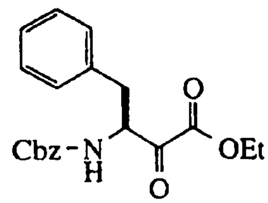
flow-through supernatant concentrated by tangential flow against a 10 K cut-off membrane. The retentate was passed over a DE52 anion exchange column (5 x 20 cm) equilibrated in the same buffer. The flow-through from this column was passed over an S-Sepharose Fast Flow matrix (2.5 x 20 cm column, Pharmacia), again equilibrated at pH 8 in the same buffer. The flow-through from S-Sepharose was made 1M with respect to ammonium sulfate and applied to a phenyl sepharose column (Pharmacia, 1.5 x 10 cm), washed with lysis buffer containing 1M ammonium sulfate, then eluted with a 100-0% linear ammonium sulfate gradient. Peak fractions containing PR were pooled, concentrated using Centripreps (Amicon), and dialyzed against 10 mM Tris-HCl, pH 8, 5 mM EDTA, 2 mM 2-mercaptoethanol. The sample was made 10 mM with respect to MOPS, adjusted to pH 5.5 with HCl, then applied to a Resource S column (Pharmacia) equilibrated in 10 mM Tris-MOPS, pH 5.5, 5 mM EDTA and 2 mM 2-mercaptoethanol. PR was eluted using a linear 0-300 mM NaCl gradient in the same buffer. Peak fractions were pooled, concentrated, and stored as aliquots at -20 °C for further studies. The integrity of the isolated FIV PR was confirmed by ion spray mass spectrometry.

2) Chemical Synthesis

All manipulations were conducted under an inert atmosphere (argon or nitrogen). All solvents were reagent grade. Anhydrous ether, tetrahydrofuran (THF), and toluene were distilled from sodium and/or benzophenone ketyl. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). *N, N,* Dimethylformamide (DMF) and acetonitrile were distilled from phosphorous pentoxide and calcium hydride. Methanol was distilled from magnesium and iodine. Organic acids and bases were reagent grade. All other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60 F-254) plates (0.25 mm). Visualization was effected using standard procedures unless otherwise stated. Flash column chromatography was carried out on Merck silica gel 60 particle size (0.040-0.063 mm, 230-400 Mesh). Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton and carbon magnetic resonance spectra (^{1}H-NMR, ^{13}C-NMR) were recorded on either a Bruker AM-500, AMX-400 or AC250MHz Fourier transform spectrometer. Coupling constants (*J*) are reported in hertz and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS, 0 ppm), MeOH (3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C) or $CHCl_3$ (7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C) as internal reference. Infrared spectra (IR) were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. Absorptions are reported in wavenumbers (cm^{-1}).

Peptide fragments described herein were synthesized using traditional peptide coupling methodologies [EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl), HOBt (1-hydroxybenzotriazole) and DIEA (diisopropylethylamine)]. Esters were hydrolyzed either by base (LiOH for methyl esters) or acid (TFA for ^{t}-butyl esters).

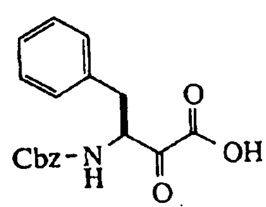
Synthesis of compound 10 as illustrated in figur 6



10

Compound 10: The α-keto ester **10** was synthesized according to the method described by Angelastro et al. *J. Org. Chem.* **1989**, *54*, 3913-3916. It is possible to synthesize α-keto esters *via* other methodologies, Wasserman, et al. *J. Org. Chem.* **1994**, *59*, 4364-4366(43); Wong et al. *J. Med. Chem* **1993**, *36*, 211-220, but the route employed was found to be most concise and high yielding. The methodology, shown in figure 1 and described by Angelastro, is as follows (i) *t*-BuLi, ethyl vinyl ether, $MgBr_2$, THF; (ii) O_3, CH_2Cl_2.

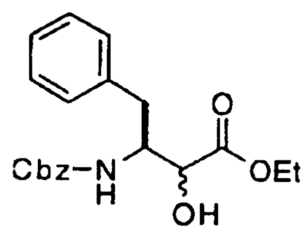
Synthesis of compound 11 as illustrated in figure 6, step (iii)



11

Compound 11. To a 2:1 solution of compound **10** in methanol/water (.10 Molar) is added a 0.17 Normal solution of LiOH in water and the reaction is allowed to stir at 0 °C for 2 hours. The mixture is then purified by reverse phase chromatography and yielded compound 11 in 98% overall yield.

Synthesis of (2*R*, 3*S*) and (2*S*, 3*S*) *N*-(Benzyloxycarbonyl)-AHAP-(3-Amino-2-Hydroxy-4-Phenylbutanoic Acid) Ethyl Ester 12 and 13 as shown in figure 7.

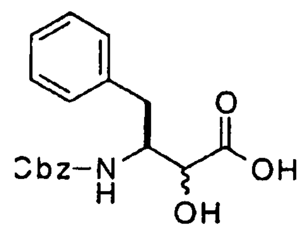


12 - *R*
13 - *S*

As illustrated in figure 7, step i, the substrate **10** (600 mg, 1.7 mmol) was dissolved in methanol (10 mL) and cooled to 0 °C. Sodium Borohydride (70.3 mg, 1.9 mmol) was then added. After 20 minutes the reaction was quenched by addition of saturated ammonium chloride$_{(aq.)}$ (10 mL). The reaction mixture was concentrated *in vacuo* to remove most of the methanol. The aqueous residue was then extracted with ethyl acetate (3 x 20 mL), washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a mixture of diastereomers. The alcohols

were separated by flash chromatography eluting with 15% ethyl acetate in hexane to give the alcohols in a ratio of 3:4, **12** & **13** (590 mg, 97%). R_f = 0.54 and 0.40 respectively (EtOAc/Hexane, 1:2): **12 (2*R*, 3*S* - colorless oil)**; ^1H NMR (500MHz, $CDCl_3$) δ 7.34-7.18 (10H, m), 5.17 (1H, d, *J* 9.5), 5.04 (2H, s), 4.43-4.38 (1H, m), 4.33 (1H, dd, *J* 4.5, 2.0), 4.15-4.08 (1H, m), 3.98-3.92 (1H, m), 3.28 (1H, d, *J* 5.0); IR (NaCl) ν_{max} 3368, 3030, 2981, 1731, 1520, 1455, 1246, 1104, 1055, 748, 699 cm^{-1}; FABHRMS (NBA) *m/e* 358.1659 ($[M+H]^+$, $C_{20}H_{23}NO_5$ requires 358.1654); (Found: C, 67.30; H, 6.50; N, 3.99. $C_{20}H_{23}NO_5$ requires C, 67.21; H, 6.49; N, 3.92%). **13 (2*S*, 3*S* - crystalline)**; ^1H NMR (500MHz, $CDCl_3$) δ 7.34-7.18 (10H, m), 5.17 (1H, d, *J* 9.5), 5.04 (2H, s), 4.43-4.38 (1H, m), 4.33 (1H, dd, *J* 4.5, 2.0), 4.15-4.08 (1H, m), 3.98-3.92 (1H, m), 3.28 (1H, d, *J* 5.0); IR (NaCl) ν_{max} 3368, 3030, 2980, 1731, 1520, 1455, 1246, 1104, 1055, 748, 699 cm^{-1}; FABHRMS (NBA) *m/e* 358.1661 ((M^+ + H), $C_{20}H_{23}NO_5$ requires 358.1654); (Found: C, 67.22; H, 6.57; N, 3.90. $C_{20}H_{23}NO_5$ requires C, 67.21; H, 6.49; N, 3.92%). m.p. 88-89°C.

Synthesis of (2*R*, 3*S*) and (2*S*, 3*S*) *N*-(Benzyloxycarbonyl)-3-Amino-2-Hydroxy-4-Phenylbutanoic Acid 14 & 15 respectively as illustrated in figure 7

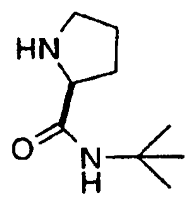


14 - *R*
15 - *S*

As illustrated in figure 7, step ii, the substrate (**12** or **13**) (250 mg, 0.70 mmol) was dissolved in 0.25 N LiOH in methanol/water, 2:1 (5 mL), and stirred at ambient temperature for 30 minutes. The pH of the reaction was adjusted to pH 7.0 with 1N HCl $_{(aq.)}$ and the methanol removed *in vacuo*. The aqueous residue was then acidified to pH 2.0 with 1N HCl $_{(aq.)}$ and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were washed with water (10 mL), brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the desired acid (**14** or **15**) as a white solid (212 mg, 92%). The acids were purified by recrystallization from hot ethanol. **14 (2*R*, 3*S*)**; ^{1}H NMR (500MHz, CD_3OD) δ 7.31-7.18 (10H, m), 6.87 (1H, d, *J* 9.5), 5.00 (1H, d, *J* 10.0), 4.96 (1H, d, *J* 10.0), 4.31-4.23 (1H, m), 4.07 (1H, d, *J* 2.5), 2.93 (1H, dd, *J* 13.5, 7.5), 2.83 (1H, dd, *J* 13.5, 8.0); ^{13}C NMR (125MHz, CD_3OD) δ 176.6 (C=O), 158.5 (C=O), 139.8 (C), 138.5 (C), 130.7 (2 x CH), 129.8 (2 x CH), 129.7 (2 x CH), 129.7 (CH), 129.1

(CH), 128.8 (CH), 127.8 (CH), 67.6 (CH), 57.1 (CH_2), 57.0 (CH_2), 39.3 (CH); FABHRMS (NBA) *m/e* 330.1353 ($[M+H]^+$, $C_{18}H_{19}NO_5$ requires 330.1341); m.p. 209-210 (decomp.). **15 (2*S*, 3*S*)**; ^1H NMR (500MHz, CD_3OD) δ 7.28-7.18 (10H, m), 7.09 (1H, d, *J* 12.5), 4.97 (1H, d, *J* 12.5), 4.92 (1H, d, *J* 12.5), 4.26 (1H, d, *J* 4.0), 4.25-4.20 (1H, m), 2.81 (1H, dd, *J* 14.0, 4.0), 2.76 (1H, dd, *J* 14.0, 4.0); ^{13}C NMR (125MHz, CD_3OD) δ 175.9 (C=O), 158.5 (C=O), 140.0 (C), 138.6 (C), 130.6 (3 x CH), 129.6 (CH), 129.5 (3 x CH), 129.0 (CH), 128.8 (CH), 127.6 (CH), 74.3 (CH), 67.4 (CH_2), 57.1 (CH), 36.5 (CH_2); FABHRMS (NBA/NaI) *m/e* 352.1174 ($[M+Na]^+$, $C_{18}H_{19}NO_5$ requires 352.1161); (Found: C, 65.34; H, 5.75; N, 4.33. $C_{18}H_{19}NO_5$ requires C, 65.64; H, 5.82; N, 4.25%); m.p. 173-174°C (decomp.).

Synthesis of L-Prolyl-*tert*-butyl amide 16 (scheme 7)



16

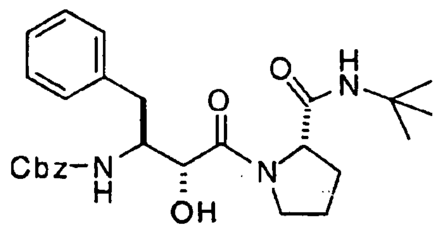
The substrate *N-tert*-Butoxycarbonyl-L-proline commercially available from Sigma, (3.0 g, 13.9 mmol), was dissolved in dry CH_2Cl_2 (20 mL). HOBT, 1-hydroxybenzotriazole hydrate (2.07 g, 15.3 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 mL, 15.3 mmol), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was diluted with ethyl acetate (100 mL), and washed with water (2 x 20 mL), 1 N HCl $_{(aq.)}$ (10 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (10 mL), water (10 mL), brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave *N-tert*-butoxycarbonyl-L-prolyl-*tert*-butyl amide as a colorless oil (1.53 mg, 40%). R_f = 0.46 (EtOAc/Hexane, 1:1). ^{1}H NMR signals broadened due to rotamers: ^{1}H NMR (500MHz, $CDCl_3$) δ 7.31-7.25 (5H, m), 6.35 (1H, br s), 4.60-4.15 (3H, m), 3.55-3.22 (2H, m), 2.41-1.70 (4H, m), 1.60-1.18 (9H, br s); IR (NaCl) ν_{max} 3298, 3086, 2976, 1698, 1660, 1531, 1398, 1162 cm^{-1}; FABHRMS (NBA/NaI) *m/e*

293.1832 ($[M+Na]^+$, $C_{14}H_{26}N_2O_3$ requires 293.1841). The *N-tert*-Butoxycarbonyl-L-prolyl-*tert*-butyl amide was carried on as follows:

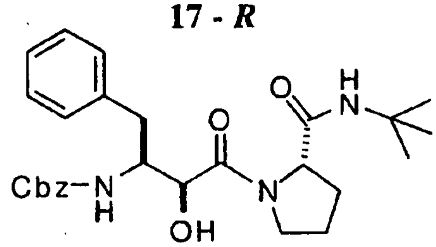
N-tert-Butoxycarbonyl-L-prolyl-*tert*-butyl amide (600 mg, 2.22 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. TFA, trifluoracetic acid, (10 mL) was then added to the solution. After 1 hour at 0 °C the reaction was concentrated *in vacuo* (any remaining TFA was removed under high vacuum) to give the trifluoroacetic acid salt of the desired amine **16** as a colorless oil (800 mg, 95%). The amine was used without further purification in subsequent coupling steps. ^1H NMR (500MHz, $CDCl_3$) δ 7.30 (1H, br s), 6.90 (1H, br s), 4.52 (1H, br s), 3.35 (2H, br s), 2.49-2.34 (1H, m), 2.08-1.97 (3H, m), 1.34 (9H, s); ^{13}C NMR (125MHz, $CDCl_3$) δ 167.4 (C=O), 59.6 (CH), 52.3 (C), 46.6 (CH_2), 30.4 (CH_2), 28.2 (3 x CH_3), 24.6 (CH_2); FABHRMS (NBA) *m/e* 171.1500 ($[M+H]^+$, $C_9H_{18}N_2O$ requires 171.1497).

General peptide coupling procedure as illustrated in figure 7:

Synthesis of (2 *S*, 3*R*) and (2*S*, 3*S*) 3-(*N*-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-*tert*-butyl amide 17 and 18.



17 - *R*



18 - *S*

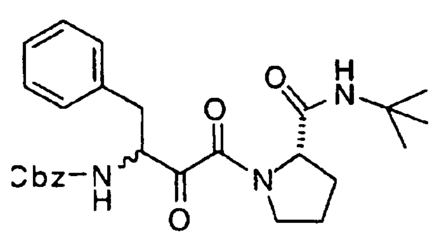
As illustrated in figure 7, step iii, the substrate **14** or **15** (70 mg, 0.213 mmol), was dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropylethylamine, (122 µl, 0.703 mmol) were added and the mixture stirred for 30 minutes at room

temperature. The secondary amine **16** as its TFA salt (73 mg, 0.255 mmol) was added and the reaction stirred for 18 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography eluting with ethyl acetate/hexane, 1:1 to give the desired coupled product **17** or **18** as a colorless oil (74 mg, 72%). R_f = 0.33 and 0.28 respectively (EtOAc:Hexane, 1:1). **17** **(2*R*, 3*S*)**; ^{1}H NMR (500MHz, $CDCl_3$) δ 7.37-7.23 (10H, m), 6.44 (1H, s), 5.16 (1H, d, *J* 9.5), 5.03 (2H, s), 4.24-4.18 (1H, m), 4.11 (1H, d, *J* 5.5), 3.97-3.90 (2H, m), 3.28-3.23 (1H, m), 3.12-3.06 (1H, m), 2.99-2.90 (2H, m), 2.18-2.12 (1H, m), 2.01-1.96 (1H, m), 1.89-1.83 (1H, m), 1.83-1.76 (1H, m), 1.27 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 171.4 (C=O), 169.9 (C=O), 156.0 (C=O), 137.4 (C), 136.7 (C), 129.2 (2 x CH), 128.7 (2 x CH), 128.4 (2 x CH), 128.0 (CH), 127.9 (2 x CH), 126.9 (CH), 68.6 (CH), 66.7 (CH_2), 61.7 (CH), 52.8 (CH), 51.1 (C), 46.1 (CH_2), 38.4 (CH_2), 28.6 (3 x CH_3), 27.4 (CH_2), 24.7 (CH_2); IR (NaCl) υ_{max} 3318, 2966, 1714, 1667, 1537, 1454, 1366, 1041 cm^{-1}; FABHRMS (NBA) *m/e* 482.2677 ([M+H]$^+$, $C_{27}H_{35}N_3O_5$ requires 482.2655); (Found: C, 67.22; H, 7.32; N, 8.80. $C_{27}H_{35}N_3O_5$ requires C, 67.34; H, 7.33; N, 8.73%). **18** **(2*S*, 3*S*)**; (major rotamer only) ^{1}H NMR (400MHz, $CDCl_3$)

δ 7.33-7.17 (10H, m), 6.45 (1H, s), 5.21 (1H, d, *J* 8.8), 4.99 (2H, s), 4.60 (1H, dd, *J* 6.8, 2.1), 4.52-4.47 (1H, m), 4.21-4.13 (1H, m), 3.81-3.66 (3H, m), 2.75-2.60 (2H, m), 2.41-2.30 (1H, m), 2.25-2.09 (1H, m), 2.05-1.90 (2H, m), 1.30 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 171.3 (C=O), 169.6 (C=O), 156.0 (C=O), 137.3 (2 x C), 129.1 (2 x CH), 128.5 (2 x CH), 128.4 (2 x CH), 128.0 (CH), 127.8 (2 x CH), 126.5 (CH), 71.3 (CH), 66.6 (CH_2), 61.1 (CH), 54.3 (CH), 51.3 (C), 47.5 (CH_2), 33.8 (CH_2), 28.6 (3 x CH_3), 26.9 (CH_2), 25.4 (CH_2); IR (NaCl) υ_{max} 3318, 2966, 1714, 1667, 1537, 1454, 1366, 1041 cm^{-1}; FABHRMS (NBA/CsI) *m/e* 614.1645 ($[M+Cs]^+$, $C_{27}H_{35}N_3O_5$ requires 614.1631); (Found: C, 67.20; H, 7.66; N, 8.54. $C_{27}H_{35}N_3O_5$ requires C, 67.34; H, 7.33; N, 8.73%).

<u>General Dess-Martin oxidation procedure as illustrated in figure 7:</u>

Synthesis of (3S) and (3*R*) 3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-*tert*-butyl amide 2.



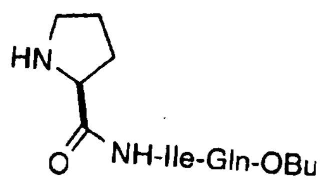
2

3:1 mixture

As illustrated in figure 7, step iv, the substrate **17** (21 mg, 0.044 mmol) was dissolved in dry CH_2Cl_2 (2 mL), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture was stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 mL) and quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 mL) and sodium thiosulfate. The aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gave the desired product **2** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%). R_f = 0.47 (EtOAc/Hexane, 1:2). Spectral data on mixture: ^{1}H NMR (400MHz, DMSO) δ 7.86 (1H, d, *J* 7.8), 7.71 (1H, d, *J* 8.3), 7.64 (1H, s), 7.53 (1H, s), 7.37-7.10 (20 H, m), 5.10 (1H, ddd, *J* 11.0, 8.3, 2.4), 5.01 (1H, d, *J* 12.6), 4.95 (1H, d, *J* 12.6), 4.95 (1H, d, *J* 16.3), 4.88 (1H, d, *J* 16.3), 4.79-4.73 (1H, m), 4.66 (1H, dd, *J* 7.8, 4.2), 4.26 (1H, dd, *J* 7.8, 4.5), 3.60-3.37 (3H, m), 3.33-3.24 (1H, m), 3.18 (1H, dd, *J* 14.7, 2.4), 3.13 (1H, dd, *J* 10.2, 3.9), 2.79 (1H, dd, *J* 13.7, 10.2), 2.46 (1H, dd, *J* 14.7, 11.0), 2.23-2.17 (1H, m), 2.04-1.97 (1H, m), 1.90-1.60 (6H, m), 1.24 (9H, s), 1.22 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 198.91 (C=O), 196.7 (C=O), 170.7 (C=O), 169.9 (C=O), 162.6 (C=O), 162.2 (C=O), 156.1 (C=O), 155.9 (C=O), 138.5 (C), 137.6 (C), 136.9 (C), 136.9 (C), 129.0 (CH x 2), 128.8 (CH x 2), 128.4 (CH x 8), 127.8 (CH x 2), 127.6 (CH x 2), 127.6 (CH x 2),

126.5 (CH), 126.4 (CH), 65.6 (CH_2), 65.3 (CH_2), 60.3 (CH), 59.7 (CH), 59.2 (CH), 58.2 (CH), 50.3 (C), 50.1 (C), 47.6 (CH_2), 47.4 (CH_2), 34.8 (CH_2), 34.1 (CH_2), 32.5 (CH_2), 29.1 (CH_2), 28.5 (CH_3 x 3), 28.4 (CH_3 x 3), 24.5 (CH_2), 21.7 (CH_2); IR (NaCl) υ_{max} 3325, 2966, 1715, 1670, 1634, 1531, 1454, 1258, 1051, 738, 699; FABHRMS (NBA/NaI) *m/e* 502.2295 ($[M+Na]^+$, $C_{27}H_{33}N_3O_5$ requires 502.2318); (Found: C, 67.62; H, 7.05; N, 8.96. $C_{27}H_{33}N_3O_5$ requires C, 67.62; H, 6.94; N, 8.76%).

Synthesis of L-Prolyl-L-isoleucyl-L-glutamine-*tert*-butyl amide 19 as illustrated in figure 8

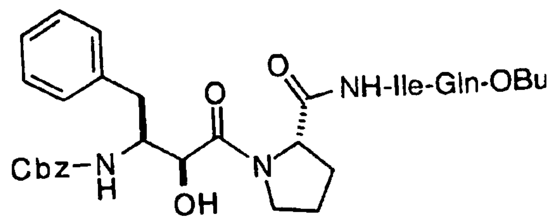


19

As illustrated in figure 8, the substrate *N-tert*-Butoxycarbonyl-L-proline commercially available from Sigma, (3.0 g, 13.9 mmol), was dissolved in dry CH_2Cl_2 (20 mL). HOBT, 1-hydroxybenzotriazole hydrate (2.07 g, 15.3 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (2.93 g, 15.3 mmol), and NH-Ile-Gln-OBu (1.6 mL, 15.3 mmol; peptide fragments were synthesized using traditional peptide coupling methodologies: EDC, HOBT, DIEA, Ile, Gln, and

protected as a tertButyl-ester), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was diluted with ethyl acetate (100 mL), and washed with water (2 x 20 mL), 1 N HCl $_{(aq.)}$ (10 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (10 mL), water (10 mL), brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave NH-Ile-Gln-OBu-L-prolyl-*tert*-butyl amide **19** as a colorless oil (1.53 mg, 40%). R_f = 0.46 (EtOAc/Hexane, 1:1). ^{1}H NMR signals broadened due to rotamers: Spectral data: ^{1}H NMR (400MHz, CD_3OD) δ 4.35-4.30 (1H, m), 4.31 (1H, dd, *J* 9.3, 5.0), 4.21 (1H, d, *J* 7.8), 3.41-3.26 (2H, m), 2.48-2.36 (1H, m), 2.36-2.22 (2H, m), 2.20-1.79 (6H, m), 1.67-1.54 (1H, m), 1.46 (9H, s), 1.32-1.17 (1H, m), 0.99 (3H, d, *J* 6.8), 0.93 (3H, t, *J* 7.4); C^{13} NMR (100MHz, CD_3OD) δ 173.3 (C=O), 172.0 (C=O), 170.1 (C=O), 167.7 (C=O), 83.0 (C), 60.7 (CH), 59.9 (CH), 54.0 (CH), 47.4 (CH_2), 37.9 (CH), 32.5 (CH_2), 31.2 (CH_2), 28.4 (CH_2), 28.2 (3 x CH_3), 26.0 (CH_2), 25.0 (CH_2), 15.9 (CH_3), 11.4 (CH_3); FABHRMS (NBA) *m/e* 435.2575 ($[M+Na]^+$, $C_{20}H_{36}N_4O_5$ requires 435.2583).

Synthesis of (2*S*, 3*S*) 3-(*N*-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-L-isoleucyl-L-glutamine-*tert*-butyl amide 20 as illustrated in figure 8

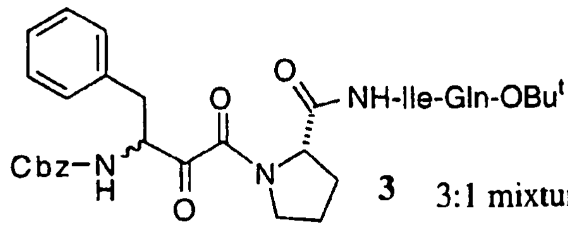


20

As illustrated in figure 8, step i, the coupling of the acid **15** to the amide **19** was carried out using the general peptide coupling procedure. A representative synthesis is as follows: the substrate **15**(70 mg, 0.213 mmol), was dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropy-lethylamine, (122 µl, 0.703 mmol) were added and the mixture stirred for 30 minutes at room temperature. The secondary amine **19** as its TFA salt (73 mg, 0.255 mmol) was added and the reaction stirred for 18 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were then washed with water (2 x 5 mL), 1 N $HCl_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and

dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography eluting with ethyl acetate/hexane, 1:1 to give the desired coupled product **20** as a colorless oil (74 mg, 72%). R_f = 0.33 and 0.28 respectively (EtOAc:Hexane, 1:1) Flash chromatography eluting with 5% methanol in dichloromethane gave the desired product **20** as a colorless oil. ^1H NMR (400MHz, $CDCl_3$) (major rotamer only) δ 7.42-7.12 (11H, m), 6.95 (1H, d, *J* 8.6), 6.79 (1H, s), 6.07 (1H, s), 5.95 (1H, d, *J* 8.3), 5.03 (1H, d, *J* 12.4), 4.96 (1H, d, *J* 12.4), 4.65 (1H, d, *J* 4.5), 4.51 (1H, dd, *J* 8.0, 5.2), 4.49-4.35 (2H, m), 4.32 (1H, d, *J* 7.8), 3.99-3.94 (1H, m), 3.70-3.50 (2H, m), 2.91-2.83 (2H, m), 2.28-2.21 (1H, m), 2.21-2.10 (3H, m), 2.02-1.75 (5H, m), 1.43 (9H, s), 1.14-1.06 (1H, m), 0.86 (3H, d, *J* 6.4), 0.78 (3H, t, *J* 7.6); C^{13} NMR (100MHz, $CDCl_3$) δ 175.5 (C=O), 172.2 (C=O), 171.5 (2 x C=O), 170.4 (C=O), 156.3 (C=O), 137.9 (C), 136.3 (C), 129.1 (2 x CH), 128.4 (4 x CH), 128.0 (CH), 127.6 (2 x CH), 126.4 (CH), 82.4 (C), 71.4 (CH), 66.5 (CH_2), 61.2 (CH), 57.8 (CH), 55.5 (CH), 52.3 (CH), 47.7 (2 x CH_2), 37.0 (CH), 33.8 (CH_2), 31.48 (CH_2), 28.76 (CH_2), 27.9 (3 x CH_3), 25.3 (CH_2), 24.8 (CH_2), 15.3 (CH_3), 11.0 (CH_3); FABHRMS (NBA/CsI) *m/e* 856.288 ($[M+Cs]^+$, $C_{38}H_{53}N_5O_9$ requires 856.2898).

(3*S*) and (3*R*) 3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-L-isoleucyl-L-glutamine-*tert*-butyl amide 3 as illustrated in figure 8

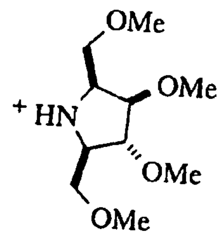


3 3:1 mixture

As illustrated in figure 8, oxidation of **20** was carried out using the general Dess-Martin oxidation procedure outlined above. Purification by flash chromatography eluting with 5% methanol in dichloromethane gave the desired α-keto amide **3(S)** (2:1 mixture of isomers) as a colorless oil (37 mg, quantitative). A representative synthesis is as follows: the substrate **20** (21 mg, 0.044 mmol) was dissolved in dry CH_2Cl_2 (2 mL), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture was stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 mL) and quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 mL) and sodium thiosulfate. The aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gave the desired product **3** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%). (S) isomer: R_f = 0.24 (EtOAc). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.38-7.16 (11H, m), 6.98 (1H, d, *J* 8.5), 6.80 (1H, s), 6.64 (1H, s), 5.64 (1H, d, *J* 8.3), 5.09 (1H, d, *J*

12.2), 5.02 (1H, d, *J* 12.2), 4.46 (1H, dd, *J* 8.2, 3.4), 4.21 (1H, t, *J* 8.1), 3.67-3.60 (1H, m), 3.59-3.45 (1H, m), 3.70-3.50 (2H, m), 3.25 (1H, dd, *J* 14.1, 5.5), 3.09 (1H, dd, *J* 14.1, 8.5), 2.29-2.12 (4H, m), 2.12-1.78 (6H, m), 1.46 (9H, s), 1.20-1.05 (1H, m), 0.93-0.83 (6H, m, 2 x CH_3); C^{13} NMR (100MHz, $CDCl_3$) δ 197.1 (C=O), 175.1 (C=O), 171.1 (C=O), 170.5 (2 x C=O), 162.9 (C=O), 156.3 (C=O), 135.7 (2 x C), 129.1 (2 x CH), 128.7 (2 x CH), 128.5 (2 x CH), 128.5 (CH), 127.5 (2 x CH), 126.9 (CH), 82.4 (C), 77.2 (CH), 67.3 (CH_2), 61.0 (CH), 58.0 (CH), 52.3 (CH), 48.0 (2 x CH_2), 37.0 (CH), 31.5 (CH_2), 29.7 (CH_2), 28.2 (CH_2), 27.9 (3 x CH_3), 25.0 (CH_2), 24.8 (CH_2), 15.5 (CH_3), 10.8 (CH_3). (3R) isomer: ^{1}H NMR (400MHz, $CDCl_3$) δ 7.38-7.16 (11H, m), 6.84 (1H, d, *J* 8.0), 6.21 (1H, s), 5.54 (1H, s), 5.51 (1H, d, *J* 8.3), 5.11-4.98 (1H, m), 5.09 (1H, d, *J* 12.5), 4.80 (1H, d, *J* 12.5), 4.75-4.61 (1H, m), 4.59 (1H, d, *J* 5.8), 4.50-4.29 (2H, m), 3.59-3.45 (1H, m), 3.29 (1H, dd, *J* 14., 3.9), 2.85 (1H, dd, *J* 14.0, 10.0), 2.29-2.12 (4H, m), 2.12-1.78 (6H, m), 1.46 (9H, s), 1.20-1.05 (1H, m), 0.93-0.83 (6H, m, 2 x CH_3). IR (NaCl) u_{max} 3290, 2925, 1728, 1648, 1537, 1452, 1367, 1247, 1157; FABHRMS (NBA/CsI) *m/e* 854.2775 ($[M+Cs]^+$, $C_{38}H_{51}N_5O_9$ requires 854.2741).

2(*R*), 5(*R*)-Bis(methoxymethyl)-3(*R*), 4(*S*)(dimethoxy)*N*pyrrolidine 23 as illustrated in figure 12



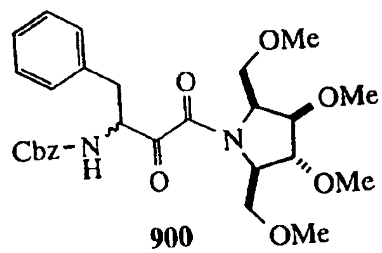
23

As illustrated in figures 11 and 12, a solution of 2(*R*), 5(*R*)-bis(hydroxymethyl)-3(*R*),4(*S*)-dihydroxypyrrolidine (806 mg, 4.95 mmol; Wong et. al J. Org. Chem. 1991, 56, 6280) in H_2O (25 mL) was cooled to 0 °C in an ice bath and the pH was adjusted to 9-10 with Na_2CO_3 solution (0.3M). Benzyloxycarbonyl chloride (1.4 mL, 9.9 mmol, 2 eq.) was added dropwise and the solution was stirred 1 hour at 0 °C, and then 1 hour at ambient temperature. Solvent was removed *in vacuo*, and the residue was taken up in EtOAc, filtered, and concentrated *in vacuo*. Flash chromatography eluting initially with 50% ethyl acetate in hexane then 100% ethyl acetate gave 2(*R*), 5(*R*)-bis(hydroxymethyl)-3(*R*), 4(*S*)-dihydroxy-*N*-(Benzyloxycarbonyl)-pyrrolidine product as a pale yellow oil.(1.1g, 81%). R_f = 0.27 (EtOAc) ^{1}H NMR (400MHz, CD_3OD) δ 7.37-7.33 (5H, m), 5.13 (2H, s), 4.20-4.19 (1H, m), 4.13-4.10 (1H, m), 4.00 (1H, br s), 3.95-3.55 (5H, m); ^{13}C NMR (100MHz, CD_3OD) (two rotamers) δ137.8, 129.6, 129.2, 129.0, 77.8, 77.1, 68.4, 67.4, 66.7, 63.2, 62.2, 61.7, 61.6, 61.1; FABHRMS (NBA) *m/e* 320.1121 ($[M+Na]^+$ $C_{14}H_{19}NO_6$ requires 320.1110).

To 2(*R*), 5(*R*)-bis(hydroxymethyl)-3(*R*), 4(*S*)-dihydroxy-*N*-(Benzyloxycarbonyl)-pyrrolidine as synthesized above (35 mg, 0.117 mmol) in dry THF (1 mL) was added CH_3I (116 µL, 1.86 mmol, 16 eq.) followed by NaH (60% dispersion in mineral oil) (28.1 mg, 6 eq.). The reaction mixture was stirred at ambient temperature for 20 h. and concentrated *in vacuo*. Flash chromatography eluting with 12% to 20% ethyl acetate in hexane gave 2(*R*), 5(*R*)-bis(methoxymethyl)-3(*R*), 4(*S*)-dimethoxy-*N*-(Benzyloxycarbonyl)-pyrrolidine as a colorless oil. (40mg, 97%). Rf = 0.6 (50% EtOAc in Hexane) ^1H NMR(400MHz, CD_3OD) δ 7.29-7.23 (5H, m), 5.04 (2H, br s), 4.10 (1H, br s), 3.74 (3H, br, s), 3.44-3.42 (4H, m), 3.34 (3H, br s), 3.31 (3H, br s), 3.23 (6H, br s); FABHRMS (NBA) *m/e* 376.1722 ($[M+Na]^+$ $C_{18}H_{27}NO_6$ requires 376.1736).

To 2(*R*), 5(*R*)-bis(methoxymethyl)-3(*R*), 4(*S*)-dimethoxy-*N*-(Benzyloxycarbonyl)-pyrrolidine as synthesized above, (40 mg, 0.181 mmol), in methanol (2 mL) was added Pd/C (10 mg). The mixture was stirred under a balloon of H_2 for 3 h. Filtration through celite followed by concentration *in vacuo* yielded the desired product **23** as a pale yellow oil (25 mg, quant.). ^1H NMR (250MHz, $CDCl_3$) δ 5.19 (1H, br s) 3.8-3.55 (8H, m), 3.54-3.35 (12H, m); ^{13}C NMR (62MHz, $CDCl_3$) 84.7, 83.7, 71.9, 69.1, 62.4, 59.9, 59.1, 59.0, 57.6, 57.5; FABHRMS (NBA) *m/e* 220.1543 ($[M+H]^+$ $C_{10}H_{21}NO_4$ requires 220.1549).

Synthesis of (3*S*) and (3*R*) 3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-[2'(*R*), 5'(*R*)-bis(methoxymethyl)-3'(*R*), 4'(*S*)-dimethoxypyrrolidine] 900 as illustrated in figure 12



3:1 mixture

900

As illustrated in figure 12, step i-ii, the substrate **15** (70 mg, 0.213 mmol), was dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropylethylamine, (122 µl, 0.703 mmol) were added and the mixture stirred for 30 minutes at room temperature. The secondary amine **23** as its TFA salt (73 mg, 0.255 mmol) was added and the reaction stirred for 18 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography eluting with ethyl

acetate/hexane, 1:1 to give the desired coupled product **26** as a colorless oil. The reaction was followed by oxidation using the general Dess-Martin oxidation procedure as given supra for compound **2** to give the desired product **900**. Flash chromatography eluting with 20% ethyl acetate in hexane gave the α-keto amide **900** (20 mg, quantitative). R_f = 0.63 (50% EtOAc in hexane). All analysis performed on mixture of isomers: NMR major isomer: ^{1}H NMR (400MHz, $CDCl_3$) δ 7.32-7.17 (10H, m), 5.75 (1H, d, *J* 8.7), 5.16-5.12 (1H, m), 5.09 (1H, d, *J* 12.4), 5.04 (1H, d, *J* 12.4), 4.39 (1H, dt, *J* 3.9, 8.3), 3.98-3.96 (1H, m), 3.92 (1H, t, *J* 6.0), 3.82-3.77 (1H, m), 3.73 (1H, dd, *J* 10.0, 5.0), 3.55 (1H, dd, *J* 10.0, 2.8), 3.47-3.17 (3H, m), 3.45 (3H, s), 3.40 (3H, s), 3.26 (3H, s), 3.19 (3H, s), 3.11 (1H, dd, *J* 14.1, 6.92); C^{13} NMR (100MHz, $CDCl_3$) δ 197.62 (C=O), 164.7 (C=O), 155.7 (C=O), 136.4 (C), 129.7 (2 x CH), 128.4 (2 x CH), 128.3 (4 x CH), 127.9 (CH), 126.7 (CH), 84.6 (CH), 83.6 (CH), 70.7 (CH_2), 69.7 (CH_2), 66.8 (CH_2), 60.7 (CH), 58.9 (CH), 58.7 (OCH_3), 58.7 (OCH_3), 58.4 (OCH_3), 58.4 (OCH_3), 56.7 (CH), 37.3 (CH_2) minor isomer: ^{1}H NMR (400MHz, $CDCl_3$) δ 7.32-7.17 (10H, m), 5.46 (1H, d, *J* 9.0), 5.11-4.87 (3H, m), 4.56-3.17 (10H, m), 3.45 (3H, s), 3.38 (3H, s), 3.31 (3H, s), 3.21 (3H, s). IR (NaCl) υ_{max} 2930, 1717, 1635, 1506, 1456, 1110; FABHRMS (NBA/CsI) *m/e* 661.1550 ($[M+Cs]^+$, $C_{28}H_{36}N_2O_8$ requires 661.1526).

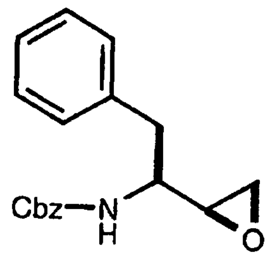
General peptide coupling procedure to form α-ketoamide from pyrolidine as illustrated in figure

12:

As illustrated in figure 12, steps i-ii, the substrate **15** (70 mg, 0.213 mmol), is dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropylethylamine, (122 µl, 0.703 mmol) are added and the mixture is stirred for 30 minutes at room temperature. The secondary amine **22; 23; 24; 16; 25; 36; 37; 40; 42; 44; 46; 47; 48; 52; 53; 54; 58; 59; 60; 64; 65; 66; 100; 101; 102 or 103** as its TFA salt (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product which is directly carried on to the next step for oxidation of the secondary alcohol as follows. The secondary alcohol (21 mg, 0.044 mmol) is dissolved in dry CH_2Cl_2 (2 mL), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture is stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 mL) and quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 mL) and sodium thiosulfate. The aqueous phase is extracted with ethyl acetate (3 x 20 mL).

The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gives the respective product **70;900;71;2;73;74;75;76;77;78;79a;80a;81a;79b;80b;81b ;82a;83a;84a;82b;83b;84b;1c;107;108 or 109** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%) as a colorless oil.

Synthesis of epoxide 21 as shown in figures 11 and **14.**



21

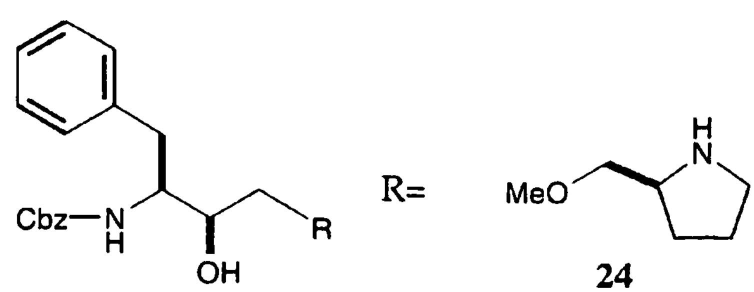
Compound 21: To a solution of compound **15** in methanol:THF (.10 M; 1:2), at 0 °C, is added a solution boran·dimethyl sulfide (5 equivalents; 1.0 M) and allowed to stir for overnight. The solvent is then removed and the mixture is resuspended in methylene chloride (.10 M) and 1.2 equivalents of triethylamine is added at 0 °C and allowed to stir for 1 hour. Next, 1.1 equivlanents of tosylchloride is added and allowed to stir at 0 °C for an additional hour. The reaction mixture is then quenched with water, extracted with 100% ethylacetate, washed over bicarbonate, brine and dried over magnesium sulfate. The crude compound is purifed by flash chromatography. The compound is then resuspended in MeOH at 0 °C and 10 equivalents of KCO_3 is added and allowed to stir for one hour. The reaction mixture is then filtered through celite, quenched with water, extracted with 100%

ethylacetate, washed over bicarbonate, brine and dried over magnesium sulfate. The crude compound is purifed by flash chromatography give compound **21**.

General procedure for coupling of epoxide to proline derivatives as illustrated in figure 11

To the pyrrolidine derivative **22; 23; 24; 16; 25; 36; 37; 40; 42; 44; 46; 47; 48; 52; 53; 54; 58; 59; 60; 64; 65; 66; 100; 101; 102 or 103** (20 mg, 0.091 mmol) was added dry methanol (2 mL), Cbz-phenylalanyl epoxide **21** (27 mg, 0.091 mmol, 1.0 eq.) and triethylamine (14 µL, 0.100mmol, 1.1eq.). The solution was refluxed for 32 h, and then concentrated *in vacuo*. Flash chromatography, eluting with ethyl acetate provides the desired product as a clear oil to give respectively hydroxylethyl amine derivatives: **400; 500; 600; 700; 800; 38; 39; 41; 43; 45; 49; 50; 51; 55; 56; 57; 61; 62; 63; 67; 68; 69; 1b; 104; 105; 106.**

Synthesis of *N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*) methoxymethyl]-pyrrolidine 600 as illustrated in figure 11

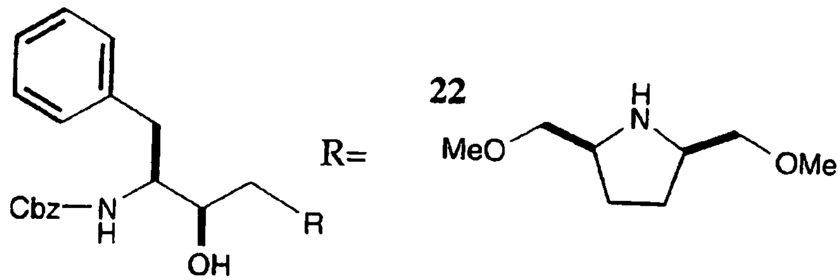


600

24

Compound 600: The pyrrolidine derivative **24** was coupled to the epoxide **21** as described in the general procedure to provide **600**. Flash chromatography eluting with ethyl acetate provided the desired product as a clear oil (40 mg, 56%). R_f = 0.15 (EtOAc). ^{1}H NMR (400MHz, CD_3OD) δ 7.29-7.16 (10H, m), 4.97 (1H, d, *J* 12.7), 4.92 (1H, d, *J* 12.7), 3.85-3.81 (1H, m), 3.77-3.73 (1H, m), 3.42-3.25 (3H, m), 3.31 (3H, s), 3.25-3.02 (2H, m), 2.89 (1H, m), 2.64 (1H, dd, *J* 13.8, 10.5), 2.61-2.52 (2H, m), 1.95-1.87 (1H, m), 1.84-1.77 (2H, m), 1.61-1.55 (1H, m); C^{13} NMR (100MHz, CD_3OD) δ 158.5 (C=O), 140.3 (2 x C), 130.5 (3 x CH), 129.4 (CH), 129.2 (3 x CH), 128.8 (CH), 128.5 (CH), 127.2 (CH), 73.0 (CH), 67.1 (2 x CH_2), 59.9 (CH_2), 59.3 (2 x CH_2), 57.5 (OCH_3), 57.2 (CH_2), 36.8 (CH_2), 28.5 (CH_2), 24.2 (CH_2); IR (NaCl) υ_{max} 3330, 2939, 1699, 1538, 1454, 1252, 1203, 1134, 699; FABHRMS (NBA/NaI) *m/e* 413.2458 ($[M+H]^+$, $C_{24}H_{32}N_2O_4$ requires 413.2440).

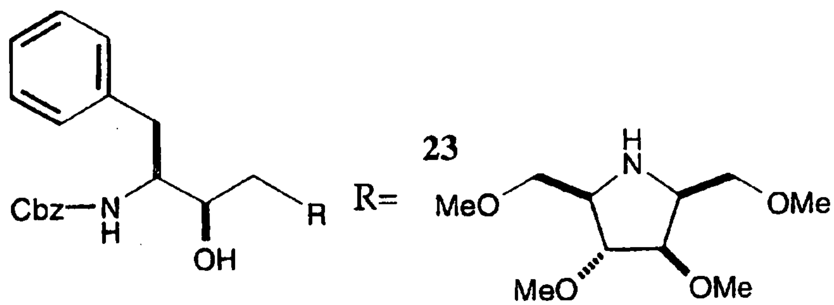
Synthesis of *N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*), 5'(*R*)-bis(methoxymethyl)]-pyrrolidine 400 as illustrated in figure 11



400

Compound 400: The pyrrolidine derivative **22** (Cignarella, G.; Nathansohn, G. *J. Org. Chem.* **1960**, *26*, 1500-1502) was coupled, according to the above conditions, to the epoxide **21** to provide **400**. Flash chromatography eluting with 30% to 50% ethyl acetate in hexane provided the desired product as a clear oil (35 mg, 40%). R_f = 0.47 (EtOAc/Hexane, 1:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.17 (10H, m), 5.02 (2H, dd, *J* 20.4, 12.1), 3.89 (1H, m), 3.50 (1H, m), 3.37 (3H, s), 3.31 (2H, d, *J* 1.1), 3.24 (3H, s), 3.20-3.18 (2H, m), 2.95-2.89 (4H, m), 2.85-2.75 (2H, m), 1.87-1.83 (2H, m), 1.54-1.52 (2H, m); ^{13}C NMR (100MHz, $CDCl_3$) δ 137.9, 129.6, 128.4, 128.2, 127.9, 127.8, 126.2, 77.5, 76.8, 71.2, 66.6, 66.3, 60.4, 59.0, 58.8, 54.9, 36.2, 29.6; FABHRMS (NBA) *m/e* 457.2689 ($[M+H]^+C_{26}H_{36}N_2O_5$ requires 457.2702)

Synthesis of *N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*), 5'(*R*)-bis(methoxymethyl)-3'(*R*), 4'(*S*)-dimethoxy]-pyrrolidine 500 as illustrated in figure 11.



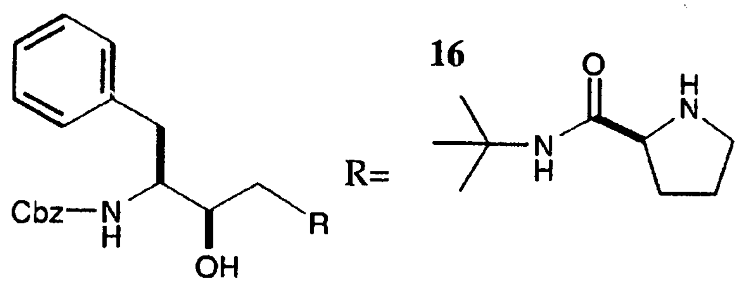
500

Compound 500: The pyrrolidine derivative **23** was coupled to the epoxide **21** as described above. Flash chromatography eluting with 50% ethyl acetate in hexane gave the desired product **500** as a pale yellow oil (20mg, 42%) R_f = 0.37 (EtOAc/Hexane, 1:1).

^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.19 (10H, m), 5.02 (2H, dd, *J* 12.3, 20.6), 4.09 (1H, br s) 3.90 (1H, m), 3.70 (1H, d, *J* 3.7), 3.58 (1H, dd, *J* 6.4, 9.3), 3.54 (2H, m), 3.37 (3H, s), 3.36 (3H, s), 3.35 (3H, s), 3.40-3.28 (5H, m), 3.28 (3H, s), 3.25-3.17 (1H, m), 2.95-2.85 (2H, m), 2.82-2.77 (2H, m); ^{13}C NMR (100MHz, $CDCl_3$) δ 155.9, 137.9, 129.7, 128.4, 128.3, 127.9, 127.8, 126.3, 84.8, 83.9, 75.1, 72.2, 70.6, 69.9, 66.9, 66.4, 61.0, 59.0, 58.9, 58.0, 57.1, 54.9, 29.7;

FABHRMS (NBA) *m/e* 517.2932 ($[M+H]^+$ $C_{28}H_{40}N_2O_7$ requires 517.2914).

Synthesis of *N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-L-prolyl-tert-butyl amide 700 as illustrated in figure 11.



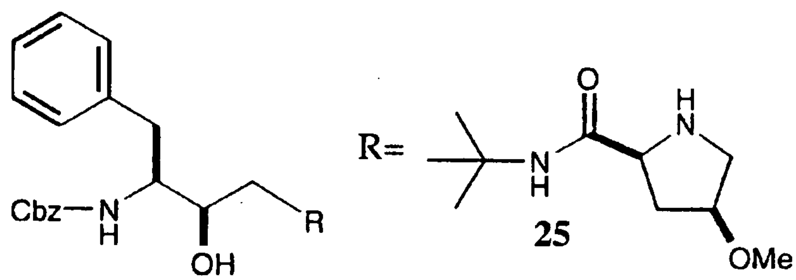
700

Compound 700: L-Proline *tert*-butyl amide **16** (synthesized supra) was coupled according to the above conditions to the epoxide **21** to give **700**. Flash chromatography eluting with 100% ethyl acetate in hexane gave the desired product **700** as a colorless oil (70mg, 48%). R_f = 0.17 (EtOAc/Hexane, 1:1).

^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.14 (10H, m), 7.02 (1H, br s), 5.26 (1H, br s), 5.06 (2H, s), 3.93-3.86 (1H, m), 3.67-3.65 (1H, m), 3.29-3.16 (1H, m), 2.90-2.75 (3H, m), 2.67 (1H, d, *J* 1.7), 2.48 (1H, dd, *J* 4.8, 2.5), 2.20-2.05 (1H, m), 1.95-1.65 (3H, m), 1.30 (9H,

s); ^{13}C NMR (63MHz, $CDCl_3$) δ 175.7, 174.4, 137.5, 129.2, 128.5, 128.4, 128.0, 127.9, 126.5, 72.1, 68.9, 66.7, 59.8, 56.1, 55.5, 50.4, 35.4, 30.9, 29.6, 28.6, 24.3; FABHRMS (NBA) *m/e* 468.2810 ($[M+H]^+$ $C_{27}H_{37}N_3O_4$ requires 467.2862).

Synthesis of *N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*)-(*tert*-butylamido)-4'(*S*)-methoxy]-pyrrolidine 800 as illustrated in figure 11.

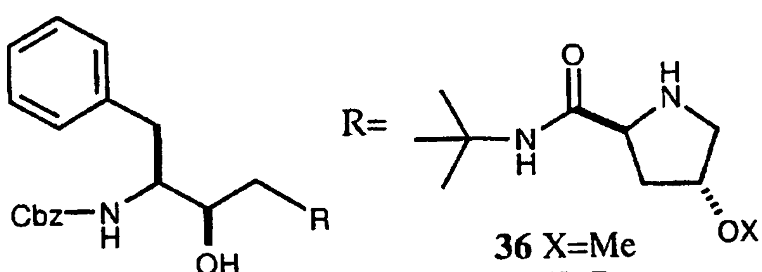


800

Compound 800: the pyrrolidine derivative **25** (derived from *cis*-4-hydroxy-L-proline) was coupled to the epoxide **21** as described above. Flash chromatography eluting with 50% ethyl acetate in hexane gave the desired product **800** as a pale yellow oil (40mg, 60%) R_f = 0.23 (EtOAc). ^1H NMR (250MHz, $CDCl_3$) δ 7.34-7.14 (10H, m), 7.04 (1H, br s), 5.01 (2H, br s,), 4.81 (1H, d, *J* 8.8), 3.98-3.85 (1H, m), 3.84 (1H, t, *J* 3.7), 3.58 (1H, dd, *J* 6.0, 6.1), 3.35-3.25 (1H, m), 3.28 (3H, s), 3.20-3.12 (1H, m), 3.00-2.90 (2H, m),

2.85 (1H, dd, J 13.3, 8.1), 2.71 (1H, d, J 12.4), 2.66 (1H, d, J 12.4), 2.56 (1H, dd, J 10.3, 3.1), 2.30-2.10 (1H, m), 2.05-1.95 (1H, m), 1.33 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 174.7 (C=O), 172.0 (C=O), 137.5 (C), 129.5 (2 x CH), 129.3 (C), 128.5 (3 x CH), 128.4 (2 x CH), 128.0 (CH), 127.9 (CH), 126.5 (CH), 79.9 (CH), 71.4 (CH), 68.3 (CH), 66.6 (C), 60.6 (CH_2), 59.7 (CH_2), 56.0 (CH_3O), 55.0 (CH), 50.3 (CH_2), 35.8 (CH_2), 29.7 (CH_2), 28.6 (3 x CH_3); FABHRMS (NBA/CsI) *m/e* 630.1970 ($[M+Cs]^+$ $C_{28}H_{39}N_3O_5$ requires 630.1944).

Synthesis of *N*-[1-Phenyl-2(*S*)-[(benzyoxy-carbonyl)amino]-3(*R*)-hydroxy-butan-4-[2'-(*S*)-(*tert*-butyl-amido)-4'(*R*)-methoxy]-pyrrolidine 38 as illustrated in figure 11.



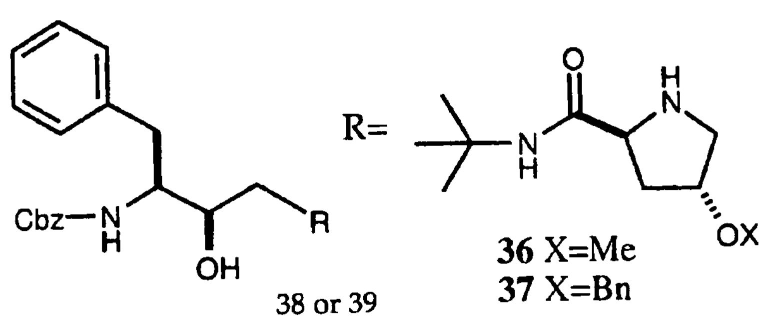
38 or 39

36 X=Me
37 X=Bn

Compound 38: The pyrrolidine derivative **36** (derived from *trans* 4-hydroxy-L-proline) was coupled to the epoxide **21** as described above. Flash chromatography eluting with 75% ethyl acetate in hexanes gave the desired product **38** as a pale yellow oil (45mg, 40%)

R_f = 0.20 (EtOAc/Hexane, 4:1).
^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.13 (10H, m), 6.79 (1H, br s), 5.02 (2H, s), 4.89 (1H, d, *J* 7.5), 3.88-3.85 (2H, m), 3.72-3.61 (1H, m), 3.49-3.33 (1H, m), 3.29 (3H, s), 3.20 (1H, t, *J* 8.0), 2.97-2.57 (5H, m), 2.29-2.22 (1H, m), 1.95-1.88 (1H, m), 1.67 (1H, br s), 1.31 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) 173.6, 156.5, 137.7, 136.3, 128.9, 128.3, 127.7, 127.6, 126.4, 79.7, 72.0, 68.0, 65.8, 60.2, 59.9, 56.6, 55.5, 50.5, 36.8, 35.3, 28.6; IR (NaCl) υ_{max} 3307, 2968, 2932, 2357, 1749, 1713, 1652, 1531, 1455, 1365, 1258, 1230, 1095, 1027, 734, 698; FABHRMS (NBA) *m/e* 498.2955 ($[M+H]^+$ $C_{28}H_{39}N_3O_5$ requires 498.2968); Found: C, 67.36; H, 8.30; N 8.49. $C_{28}H_{39}N_3O_5$ requires C, 67.57; H, 7.90; N, 8.45).

N-[1-Phenyl-2(*S*)-[(benzyoxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*S*)-(*tert*-butylamido-4'(*R*)-benzyloxy]-pyrrolidine 39 as illustrated in figure 11.

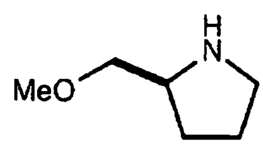


38 or 39

Compound 39: The pyrrolidine derivative **37** (derived from *trans* 4-hydroxy-L-proline) was coupled to the

epoxide **21** as described above. Flash chromatography eluting with 50% ethyl acetate in hexanes gave the desired product **39** as colorless oil (28mg, 53%) R_f = 0.20 (EtOAc/ Hexane, 1:1). ^{1}H NMR(400MHz, $CDCl_3$) 7.34-7.16 (15H, m), 6.76 (1H, br s), 5.01 (2H, s), 4.85 (1H, d, *J* 8.2), 4.51 (1H, d, *J* 11.7), 4.43 (1H, d, *J* 11.7), 4.13-4.07 (1H, m), 3.90-3.80 (1H, m), 3.70-3.62 (1H, m), 3.50-3.35 (2H, m), 3.24 (1H, t, *J* 8.0), 2.92-2.68 (4H, m), 2.35-2.29 (1H, m), 1.99-1.93 (1H, m), 1.63 (1H, br s), 1.30 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) 137.5, 129.3, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 126.6, 80.1, 77.9, 71.1, 68.2, 60.8, 60.1, 55.7, 37.4, 35.9, 29.7, 28.6; IR (NaCl) υ_{max} 3307, 2923, 1713, 1652, 1532, 1455, 1365, 1263, 1229, 1097, 1028, 733, 699; FABHRMS (NBA) *m/e* 706.2288 ($[M+Cs]^+$ $C_{34}H_{43}N_3O_5$ requires 706.2257).

Synthesis of compound 24 as shown in figure 11; 12

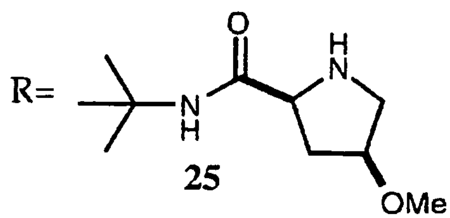


24

Compound 24: To a solution of L-proline in methanol:THF (.10 M; 1:2), at 0 °C, is added a solution boran·dimethyl sulfide (5 equivalents; 1.0 M) and allowed to stir for overnight. The solvent is then removed and the mixture is resuspended in methylene chloride (.10 M) and 1.2 equivalents of NaH (30%) is added at 0 °C and allowed to stir for 1 hour. Next, 1.1 equivlanents of methyliodide is

added and allowed to stir at 0 °C for an additional hour. The reaction mixture is then quenched with water, extracted with 100% ethylacetate, washed over bicarbonate, brine and dried over magnesium sulfate. The crude compound is purifed by flash chromatography to give compound **24**.

Synthesis of compound 25 as shown in figure 11; 12

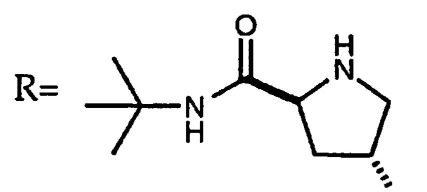


25

Compound 25: The substrate *cis* 4-hydroxy-L-proline commercially available from Sigma, (3.0 g, 13.9 mmol), was dissolved in dry CH_2Cl_2 (20 mL). HOBT, 1-hydroxybenzotriazole hydrate (2.07 g, 15.3 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 mL, 15.3 mmol), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was diluted with ethyl acetate (100 mL), and washed with water (2 x 20 mL), 1 N HCl $_{(aq.)}$ (10 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (10 mL), water (10 mL), brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave *N*-*tert*-butoxycarbonyl-*cis*-4-hydoroxy-L-prolyl-*tert*-butyl amide as a colorless

oil. The compound was then resuspended in metylene chloride (.10 M) and 1.2 equivalents of NaH (30%) is added at 0 °C and allowed to stir for 1 hour. Next, 1.1 equivlanents of methyliodide is added and allowed to stir at 0 °C for an additional hour. The reaction mixture is then quenched with water, extracted with 100% ethylacetate, washed over bicarbonate, brine and dried over magnesium sulfate. The crude compound is purifed by flash chromatography to give compound **25**.

Synthesis of compound 36 as shown in figure 11; 12

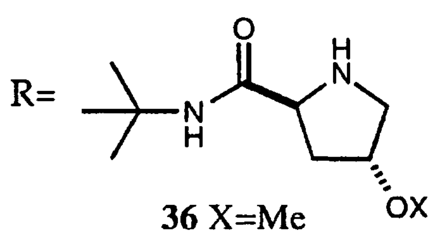


36 X=Me
37 X=Bn

Compound 36: The substrate *trans*- 4-hydroxy-L-proline commercially available from Sigma, (3.0 g, 13.9 mmol), was dissolved in dry CH_2Cl_2 (20 mL). HOBT, 1-hydroxybenzotriazole hydrate (2.07 g, 15.3 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 mL, 15.3 mmol), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was diluted with ethyl acetate (100 mL), and washed with water (2 x 20 mL), 1 N HCl $_{(aq.)}$ (10 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (10 mL), water (10 mL), brine (10 mL) and dried ($MgSO_4$) before

concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave *N-tert*-butoxycarbonyl-*trans*-4-hydoroxy-L-prolyl-*tert*-butyl amide as a colorless oil. The compound was then resuspended in metylene chloride (.10 M) and 1.2 equivalents of NaH (30%) is added at 0 °C and allowed to stir for 1 hour. Next, 1.1 equivlanents of methyliodide is added and allowed to stir at 0 °C for an additional hour. The reaction mixture is then quenched with water, extracted with 100% ethylacetate, washed over bicarbonate, brine and dried over magnesium sulfate. The crude compound is purifed by flash chromatography to give compound **36**.

Synthesis of compound 37 as shown in figure 11; 12

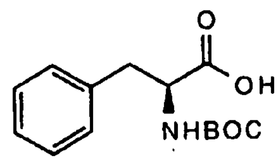


36 X=Me
37 X=Bn

Compound 37: The substrate *trans*- 4-hydroxy-L-proline commercially available from Sigma, (3.0 g, 13.9 mmol), was dissolved in dry CH_2Cl_2 (20 mL). HOBT, 1-hydroxybenzotriazole hydrate (2.07 g, 15.3 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 mL, 15.3 mmol), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was

diluted with ethyl acetate (100 mL), and washed with water (2 x 20 mL), 1 N HCl $_{(aq.)}$ (10 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (10 mL), water (10 mL), brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave *N*-*tert*-butoxycarbonyl-*trans*-4-hydoroxy-L-prolyl-*tert*-butyl amide as a colorless oil. The compound was then resuspended in methylene chloride (.10 M) and 1.2 equivalents of NaH (30%) is added at 0 °C and allowed to stir for 1 hour. Next, 1.1 equivlanents of benzylbromide is added and allowed to stir at 0 °C for an additional hour. The reaction mixture is then quenched with water, extracted with 100% ethylacetate, washed over bicarbonate, brine and dried over magnesium sulfate. The crude compound is purifed by flash chromatography to give compound **37**.

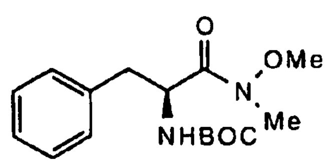
Synthesis of compound 27 (compound is shown in figures 9 and 10)



27

Compound 27: To a solution of phenylalanine (1.0 equiv.) in acetonitrile (.10 Molar) was added 1.2 equivalents of commercially available BOC-ON [2-(tert-butoxycarbonyloxyimino)-2-phenyl-acetonitrile] and the mixture was allowed to stir for 12 hours at 25 °C. The solvent was then evaporated and the crude compound **27** was carried on to the next step.

Synthesis of compound 28 as illustrated in figure 9



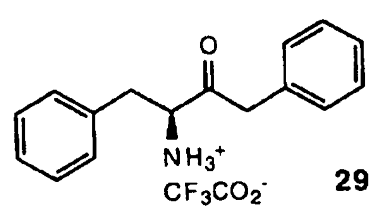
94%

28

Compound 28: The substrate **27** (70 mg, 0.213 mmol), was dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43

mg, 0.224 mmol), DIEA, diisopropyl-ethylamine, (122 µl, 0.703 mmol) were added and the mixture stirred for 30 minutes at room temperature. The commercially available N-methoxy-methyl amine hydrochloride salt (73 mg, 0.255 mmol; Aldrich) was added and the reaction stirred for 18 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography eluting with ethyl acetate/hexane, 1:1 to give the desired coupled product **28** as a colorless oil (94%).

Synthesis of 3(S)-1,4-Diphenyl-2-oxo-3-amino-N-Boc-butane (29) as illustrated in figure 9



29

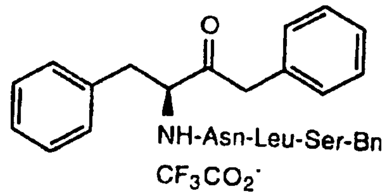
70%

3(S)-1,4-Diphenyl-2-oxo-3-amino-N-Boc-butane (29): To a stirred solution of N-Boc-L-phenylalanine-N-methoxy-N-methylamide (28) (5.0 g, 14.5 mmol) in anhydrous THF (50 mL) under N_2 at 0°C was added 2.0 M

benzyl magnesium chloride in THF (21.7 mL, 43.5 mmol). The mixture was gradually warmed at room temperature and stirred for an additional 3h. The reaction mixture was then poured onto 1 N HCl (25 mL). The organic layer was separated and the aqueous layer was extracted with ether (3 x 35 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give a crude product. Purification of the crude material by flash chromatography (EA:H;1:4) afforded **Boc protected intermediate to 29** as a white solid (4.8 g, 98%). R_f 0.3 (EA:H;1:4); mp 86-87°C; $[\alpha]^{25D}$ +31.22° (c 2.21, CH_2Cl_2); IR 3485, 2978, 1709, 1704, 1490, 1363, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) 1.14 (s, 9H), 2.9-3.15 (m, 2H), 3.65 (q, 2H, J=11.6 Hz), 4.61 (d, 1H, J=6.9 Hz), 5.1 (bs, 1H), 7.0-7.2 (m, 10H) ppm; ^{13}C NMR ($CDCl_3$) 28.3, 37.8, 47.8, 59.5, 79.9, 127.0, 127.1, 128.8, 129.2, 129.2, 129.6, 133.1, 135.2, 155.1, 206.5 ppm. HRMS: 472.0880, Calcd for $C_{21}H_{25}NO_3+Cs^+$: 472.0889.

3(S)-1,4-Diphenyl-2-oxo-3-amino-butane HCl (29): To a solution of 3(S)-1,4-diphenyl-2-oxo-3-amino-N-Boc-butane (**Boc protected intermediate to 29**) (1.4 g, 4.12 mmol) in ether (10 mL) was added a saturated solution of HCl(g)/Ether (20 mL). After 3 h the precipitate was filtered to afford a crude white solid. Recrystallization (MeOH/Ether) gave **29** as a white solid (0.96 g, 85%). ^1H NMR (300 MHz, CD_3OD) 2.96 (dd, 1H, J=8.2 Hz), 3.24 (dd, 1H, J=6.2, 14.3 Hz), 3.73 (q, 2H, J=16.9 Hz), 4.41 (dd, 1H, J=2.0, 8.2 Hz) ppm. HRMS: 240.1388 Calcd. for $C_{16}H_{18}NO + H^+$: 240.1388.

Synthesis of Compound 30 as illustrated in figure 9



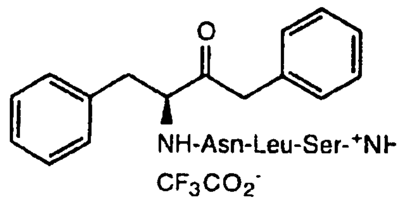
30

Compound 30: To a solution of **29** (0.032 g, 0.12 mmol) in CH_2Cl_2 (10 mL) was added O-benzyl-N-Boc-L-Ser-Leu-Asn (0.075 g, 0.15 mmol), EDC (0.032 g, 0.17 mmol), HOBt (0.045 g, 0.33 mmol) and DMAP (cat.). After 24 h the reaction mixture was washed with sat. $NaHCO_3$ (2 x 5 mL), 1 N HCl (2 x 5 mL), sat. NaCl (2 x 5 mL), dried ($MgSO_4$) and concentrated to give a crude solid. Recrystallization from MeOH/Ether afforded **intermediate N-Boc protected** compound to **30** as a white solid (0.05 g, % yield). ^1H NMR (300 MHz, DMSO-d_6) 0.72-0.85 (m, 6H), 1.35 (s, 9H), 1.50-1.72 (m, 1H), 2.30-2.45 (m, 1H), 2.80-2.90 (m, 1H), 3.05 (dd, 1H, J=5.1, 13.9 Hz), 3.5-3.63 (m, 2H), 3.69 (d, 1H), J=17.0 Hz), 3.86 (d, 1H, J=17.3 Hz), 4.20-4.30 (m, 1H), 4.30-4.45 (m, 1H), 4.46 (s, 2H), 4.48-4.51 (m, 1H), 6.80-7.42 (m, 15H) ppm. HRMS: 876.2955 Calcd for $C_{41}N_{53}N_5O_8$ + CS^+: 876.2948. Anal Cacld for $C_{41}H_{53}N_5O_8$: C 60.20%, H 5.615, N 7.18%, S 9.41%. Found C 65.99%, H 7.22%, N 9.61%.

To a solution of **intermediate N-Boc protected** compound (0.12 g, 0.16 mmol) in CH_2Cl_2 was added a solution of 305 TFA/CH_2Cl_2. After 24 h the mixture was concentrated to afford a crude white solid which after recrystallization (MeOH/Ether) afforded the

title compound **30** as a white solid (0.09 g, 75%). ^{1}H NMR (300 MHz, CD_3OD) 0.71-0.85 (m, 6H), 1.40-1.60 (m, 3H), 2.40-.262 (m, 2H), 2.70-.291 (m, 1H), 2.91-3.01 (m, 1H), 3.50-3.58 (m, 1H), 3.60-3.72 (m, 2H), 3.75-3.82 (m, 1H), 3.88-4.00 (m, 1H), 4.20-4.30 (m, 1H), 4.44-4.61 (m, 2H), 6.93-7.45 (m, 15H) ppm. HRMS: 644.3448 Calcd for $C_{36}H_{46}N_5O_6 + H^{+}$: 644.3448.

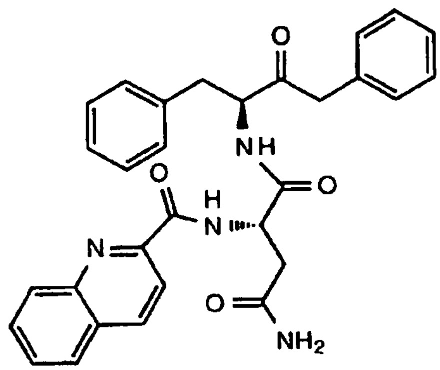
Synthesis of Compound 31 as illustrated in figure 9



31

Compound 31: A solution of **30** (80 mg, 0.11 mmol) in glacial acetic (10 mL) containing $Pd(OH)_2/C$ (cat.) was placed under a H_2 atmosphere at 50 psi. After 12 h the solution was filtered and the crude solid was recrystallized from MeOH/Ether to afford the title compound (15 mg, 22%). ^1H NMR (300 MHz, CD_3OD) 0.82 (t, 6H, J=6.6 Hz), 1.39 (t, 2H, J=7.2 Hz), 1.50-1.60 (m, 1H), 2.36 (dd, 1H, J=8.1, 15.6 Hz), 2.44-2.50 (m, 1H), 2.38 (dd, 1H, J=4.5, 13.9 Hz), 3.05 (dd, 1H, J=4.9, 13.9 Hz), 3.69 (d, 1H, J=17.3 Hz), 3.85 (dd, 1H, J=17.2 Hz), 4.28-4.32 (m, 1H), 4.35-4.42 (m, 1H), 4.48-4.53 (m, 1H), 6.80-7.35 (m, 10H) ppm. HRMS: 686.1961 Calcd for $C_{31}H_{43}N_5O_8$ + CS^+: 686.1955.

Synthesis of Compound 32 as illustrated in figure 9

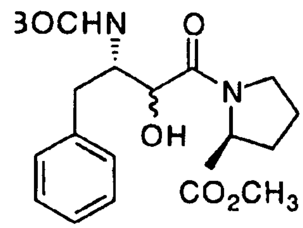


32

Compound 32: To a solution of **29** (0.17 g, 0.62 mmol) in DMF (5 mL) was added HOBt (0.17, 1.2 mmol), DMAP (cat.), EDC (0.12 g, 0.62 mmol), Et_3N (0.06 g, 0.09 mL, 0.62 mmol) and quinolinic-Asn (g, 0.52 mmol). After 12 h the reaction mixture was taken up in EA (50 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a crude brown solid. Recrystallization from MeOH/ether gave a white solid as **32** (0.12 g, 43%). ^1H NMR (300 MHz, DMSO-d_6) 2.62 (dd, 1H, J=4.9, 15.3 Hz), 2.75 (dd, 1H, J=6.9, 15.5 Hz), 2.84 (dd, 1H, J=14.3, 23.4 Hz), 3.06 (dd, 1H, J=4.6, 13.7 Hz), 3.76 (d, 1H, J=17.0 Hz), 3.91 (d, 1H, J=17.0 Hz), 4.51 (q, 1H, J=7.7 Hz), 4.82 (q, 1H, J=6.8 Hz), 6.95-7.32 (m, 11H), 7.48 (s, 1H), 7.73 (t, 1H, J=7.1 Hz), 7.89 (t, 1H, J=8.3 Hz), 8.13 (q, eH, J=8.7 Hz), 8.58 (t, 2H, J=6.7 Hz) ppm. HRMS:

641.1166 Calcd for $C_{30}H_{28}NO_4$ + Cs^+: 641.1165.

Synthesis of Compound 34 as illustrated in figure 10

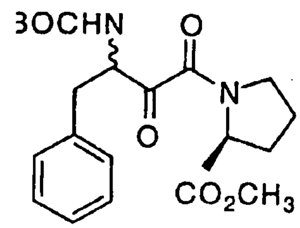


34

Compound 34: To a solution of compound **33** (7:3 mixture) (0.21 g, 0.71 mmol), as previously prepared from **27** (Yuan, W.; Munoz, B.; Wong, C.-H.; Haeggstrom, J.Z.; Wetterholm, A.; Samuelsson, B. *J. Med. Chem.* **1993**, *36*, 211) in CH_2Cl_2 (10 mL) was added L-proline methyl ester HCl (0.18 g, 1.1 mmol), EDC (0.16 g, 0.85 mmol), HOBt (0.21 g, 1.56 mmol) and DMAP (cat.). After 24 h the reaction mixture was washed with sat. $NaHCO_3$ (2 x 2 mL), 1 N HCl (2 x 2 mL) and sat. NaCl (1 x 1 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to give a crude solid. Purification by flash chromatography (EA:H; 1:4) gave the white solid compound **34** as a single (2R, 3S) (0.23 g, 80%). R_f=0.20 (EA:H;1:4); ^{1}H NMR (300 MHz, $CDCl_3$) 1.34 (s, 9H), 1.90-2.11 (m, 4H), 2.88-2.91 (m, 2H), 3.11-3.20 (m, 1H), 3.36 (q, 1H, J=7.6 Hz), 3.65 (s, 3H), 3.89 (d, 1H, J=5.1 Hz), 4.08 (d, 1H, J=5.7 Hz), 4.15 (q, 1H, J=9.7 Hz), 4.39 (d, 1H, J=7.4 Hz), 4.89 (d, 1H, J=9.9 Hz), 7.10-7.35 (m,

5H) ppm. HRMS: 539.1169 Calcd for $C_{21}H_{30}N_2O_6$ + Cs^+: 539.1158.

Synthesis of Compound 35 as illustrated in figure 10



35 65%

Compound 35: To a solution of compound **34** (0.062 g, 0.15 mmol) in anhydrous CH_2Cl_2 under N_2 at room temperature was added the Dess-Martin reagent (0.078 g, 0.18 mmol). After 12 h the reaction was quenched with sat. $NaHCO_3$ (4 mL) and sat. $Na_2S_2O_3$ (4 mL). Ether (40 mL) was added to the reaction and then stirred for 10 min. The reaction mixture was washed with sat. NaCl (2 x 10 mL), dried ($MgSO_4$), filtered and concentrated to give a crude oil. Purification by flash chromatography (EA:H; 1:3) afforded compound **35** as a 1:0.8 mixture of an oil (0.030 g, 65%). ^1H NMR (300 MHz, $CDCl_3$) 1.35; 1.35 (s;s, 9H), 1.91-2.01 (m, 3H), 2.15-2.23 (m, 1H), 3.09-3.41 (m, 1H), 3.51-3.70 (m, 2H), 3.70 (m, 2H), 3.70 (q, 3H, J=6.3, 7.8 Hz), 4.40-4.49 (m, 1H), 4.72d-4.85 (m, 1H), 4.93-5.00 (m, 1H), 5.10-5.23 (m, 1H), 7.05-7.35 (m, 5H) ppm. HRMS: 537.1002 Calcd for $C_{21}H_{28}N_2O_6$ + Cs^+: 537.1002.

General peptide coupling procedure to form α-ketoamide from piperidine as illustrated in figure 13:

As illustrated in figure 13, steps i-ii, the substrate **15** (70 mg, 0.213 mmol), is dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropylethylamine, (122 µl, 0.703 mmol) are added and the mixture is stirred for 30 minutes at room temperature. The secondary amine **85;86;87;88;89;90;91;92;93;94;95;96 or 97** as its TFA salt (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product which is directly carried on to the next step for oxidation of the secondary alcohol as follows. The secondary alcohol (21 mg, 0.044 mmol) is dissolved in dry CH_2Cl_2 (2 mL), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture is stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 mL) and quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 mL) and sodium thiosulfate. The aqueous phase is

extracted with ethyl acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gives the respective product **98; 99; 120; 121; 122; 123; 124; 125; 126; 127; 128; 129 or 130** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%) as a colorless oil.

General procedure for coupling of epoxide to proline derivatives as illustrated in figure 14

To the pyrrolidine derivative **85;86;87;88;89;90;91;92;93;94;95;96 or 97** (20 mg, 0.091 mmol) was added dry methanol (2 mL), Cbz-phenylalanyl epoxide **21** (27 mg, 0.091 mmol, 1.0 eq.) and triethylamine (14 µL, 0.100mmol, 1.1eq.). The solution was refluxed for 32 h, and then concentrated *in vacuo*. Flash chromatography, eluting with ethyl acetate provides the desired product as a clear oil to give respectively hydroxylethyl amine derivatives: **131; 132; 133; 134; 135; 136; 137; 138; 139; 140; 141; 142 or 143.**

Synthesis of compound 1001 as illustrated in Figure 15: A typical acylation is as follows: Compound **1000** (0.16 mmol; cis-dihydroxymethyl-pyrolidine from Aldrich followed by standard BOC protection; vida supra), the vinyl acetate (0.05 mL; Aldrich), DMF (0.2 mL; other solvents such as methylene chloride, chloroform, acetonitrile, tert-butyl alcohol, 3-methyl-3-pentanol, DMSO and THF can also be used),

H_2O (0.005 mL), triethylamine (0.005 mL) and subtilisin BPN' or subtilisin Carlsberg (5 mg, 50U) is stirred at 37°C for 36 h. The reaction is worked up by adding EtOAc and filtering the reaction mixture through celite. The filtrate is washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. Some of the product, if possible, is crystallized out at this stage by addition of diisopropyl ether. The mother liquor is then chromatographed (SiO_2, hexane/EtOAc, 9/1-1/1) to give the product **1001**.

Synthesis of compound 1002 as illustrated in Figure 15: Compound **1002** was disolved in 0.10 Molar solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1 equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1002**.

Synthesis of compound 1003 as illustrated in Figure 15: The substrate **1002** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium

chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 1004 as illustrated in Figure 15: Compound **1003** was disolved in 0.10 Molar solution of methanol and then 0.10 equivalents sodium methoxide was added at 0 ° C and the mixture was allowed to stir for 2 hours. The reaction is concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1004**.

Synthesis of compound 1005 as illustrated in Figure 15: Compound **1004** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (2 mL) at 0 °C under argon was added NaH (60% disp. in mineral oil) (96 mg, 2.40 mmol, 2.2 eq.). The mixture was allowed to stir for 20 min., and then benzyl bromide or methyl iodide (0.14 mL, 1.20 mmol, 1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and

concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product **1005**.

Synthesis of compound 46; 47 or 48 as illustrated in Figure 15: Compound 46; 47 or 48: To a solution of CBZ-protected amine (0.092 mmol) in MeOH (2 mL) was added 20% palladium hydroxide on carbon (10 mg) and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product.

Synthesis of compound 1007 as illustrated in Figure 16: A typical acylation is as follows: Compound **1006** (0.16 mmol; trans-dihydroxymethyl-pyrolidine from Aldrich followed by standard BOC protection; vida supra), the vinyl acetate (0.05 mL; Aldrich), DMF (0.2 mL; other solvents such as methylene chloride, chloroform, acetonitrile, tert-butyl alcohol, 3-methyl-3-pentanol, DMSO and THF can also be used), H_2O (0.005 mL), triethylamine (0.005 mL) and subtilisin BPN' or subtilisin Carlsberg (5 mg, 50U) is stirred at 37°C for 36 h. The reaction is worked up by adding EtOAc and filtering the reaction mixture through celite. The filtrate is washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. Some of the product, if possible, is crystallized out at this stage by addition of diisopropyl ether. The mother liquor is then chromatographed (SiO_2,

hexane/EtOAc, 9/1-1/1) to give the product **1007**.

Synthesis of compound 1008 as illustrated in Figure 16: Compound **1008** was disolved in 0.10 Molar solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1 equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1008**.

Synthesis of compound 1009 as illustrated in Figure 16: The substrate **1008** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N $HCl_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 1010 as illustrated in Figur 16: Compound **1009** was disolved in 0.10 Molar solution of methanol and then 0.10 equivalents sodium methoxide was added at 0 ° C and the mixture was allowed to stir for 2 hours. The reaction is concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1010**.

Synthesis of (±) compound 1011 as illustrated in Figure 16: Compound **1010** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (2 mL) at 0 °C under argon was added NaH (60% disp. in mineral oil) (96 mg, 2.40 mmol, 2.2 eq.). The mixture was allowed to stir for 20 min., and then benzyl bromide or methyl iodide (0.14 mL, 1.20 mmol, 1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product **1011**.

Synthesis of compound 52; 53 or 54 as illustrated in Figure 16: Compound 52; 53 or 54: To a solution of CBZ-protected amine (0.092 mmol) in MeOH (2 mL) was added 20% palladium hydroxide on carbon (10 mg) and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min.,

TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product.

Synthesis of compound 1013 as illustrated in Figure 17: Compound **1012** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (0.10 Molar) at 0 °C under argon was added sodium hydroxide (0.10 equivalents), followed by CBZ-Cl (1.1 equivalents; Aldrich) and the mixture was stirred at 0 °C for 20 min. and then quenched with a few drops of water. Saturated ammonium chloride solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then neutralized with saturated sodium bicarbonate solution, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product **1013**.

Synthesis of compound 1014 as illustrated in Figure 17: To a solution of substrate **1013** (0.5 mmol) in anhydrous DMF (5 mL) was added TBDPSCl (1.05eq: Aldrich), Et_3N (1.1 eq), and a catalytic amount of imidazole. The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H_2O (30 mL). The organic layer was then washed with sat'd $NH_4Cl_{(aq.)}$ solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield product. The mother liquor is then chromatographed to give the product **1014**.

Synthesis of compound 1015 as illustrated in Figure 17: To a solution of substrate **1014** (0.5 mmol) in anhydrous pyridine (5 mL) was added acetic anhydride (1.05eq: Aldrich). The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H_2O (30 mL). The organic layer was then washed with sat'd $NH_4Cl_{(aq.)}$ solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield product. The mother liquor is then chromatographed to give the product **1015**.

Synthesis of compound 1016 as illustrated in Figure 17: The substrate was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted to give the product.

Synthesis of compound 1017 as illustrated in Figure 17: Compound **1016** was disolved in 0.10 Molar solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1 equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1017**.

Synthesis of compound 1018 as illustrated in Figure 17: The substrate **1017** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 44 as illustrated in Figure 17: Step (1) Compound **1018** was disolved in 0.10 Molar solution of methanol and then 0.10 equivalents sodium methoxide was added at 0 ° C and the mixture was allowed to stir for 2 hours. The reaction is concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the CBZ-protected amine. Step (2) To a solution of CBZ-protected amine (0.092 mmol) in MeOH (2 mL) was added 20% palladium hydroxide on carbon (10 mg) and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The

suspension was filtered through a pad of celite and concentrated in vacuo to yield product compound **44**.

Synthesis of compound 1019 as illustrated in Figure 18: Compound **1012** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (0.10 Molar) at 0 °C under argon was added sodium hydroxide (0.10 equivalents), followed by CBZ-Cl (1.1 equivalents; Aldrich) and the mixture was stirred at 0 °C for 20 min. and then quenched with a few drops of water. Saturated ammonium chloride solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then neutralized with saturated sodium bicarbonate solution, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product **1019**.

Synthesis of compound 1020 as illustrated in Figure 18: To a solution of substrate **1019** (0.5 mmol) in anhydrous DMF (5 mL) was added TBDPSCl (1.05eq: Aldrich), Et_3N (1.1 eq), and a catalytic amount of imidazole. The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H_2O (30 mL). The organic layer was then washed with sat'd $NH_4Cl_{(aq.)}$ solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield product. The mother liquor is then chromatographed to give the product **1020**.

Synthesis of compound 1021 as illustrated in Figure 18: To an ice-cooled solution of substrate (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture was allowed to stir for 20 min., and then methyl iodide or benzyl bromide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford product. The mother liquor is then chromatographed to give the product **1021**.

Synthesis of compound 1022 as illustrated in Figure 18: The substrate was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted to give the product.

Synthesis of compound 1023 as illustrated in Figure 18: Compound **1022** was disolved in 0.10 Molar solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1 equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother

liquor is then chromatographed to give the product **1023**.

Synthesis of compound 1024 as illustrated in Figure 18: The substrate **1023** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 40 as illustrated in Figure 18: CBZ-protected amine **1024** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product compound **40**.

Synthesis of compound 1025 as illustrated in Figure 19: Compound **1025** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (0.10 Molar) at 0 °C under argon was added BOC-ON (1.1 equivalents; Aldrich) and the mixture was stirred at 0 °C for 20 min. and then quenched with a few drops of water. Saturated ammonium chloride solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then neutralized with saturated sodium bicarbonate solution, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product **1025**.

Synthesis of compound 1026 as illustrated in Figure 19: To a solution of substrate **1025** (0.5 mmol) in anhydrous DMF (5 mL) was added TBDPSCl (1.05eq: Aldrich), Et_3N (1.1 eq), and a catalytic amount of imidazole. The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H_2O (30 mL). The organic layer was then washed with sat'd $NH_4Cl_{(aq.)}$ solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield product. The mother liquor is then chromatographed to give the product **1026**.

Synthesis of compound 1027 as illustrated in Figure 19: To an ice-cooled solution of substrate (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture

was allowed to stir for 20 min., and then methyl iodide or benzyl bromide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford product. The mother liquor is then chromatographed to give the product **1027**.

Synthesis of compound 1028 as illustrated in Figure 19: The substrate was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted to give the product.

Synthesis of compound 1029 as illustrated in Figure 19: Compound **1028** was disolved in 0.10 Molar solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1 equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1029**.

Synthesis of compound 1030 as illustrated in Figure 19: The substrate **1029** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 42 as illustrated in Figure 19: CBZ-protected amine **1030** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product compound **42**.

Synthesis of compound 1032 as illustrated in Figure 20: Compound **1031** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (0.10 Molar) at 0 °C under argon

was added CBZ-Cl (1.1 equivalents; Aldrich) and the mixture was stirred at 0 °C for 20 min. and then quenched with a few drops of water. Saturated ammonium chloride solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then neutralized with saturated sodium bicarbonate solution, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product. Next, to a solution of substrate (0.5 mmol) in anhydrous DMF (5 mL) was added TBDMSCl (1.05eq: Aldrich), Et_3N (1.1 eq), and a catalytic amount of imidazole. The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H_2O (30 mL). The organic layer was then washed with sat'd $NH_4Cl_{(aq.)}$ solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield product. The mother liquor is then chromatographed to give the product **1032**.

Synthesis of compound 1033 as illustrated in Figure 20: The substrate **1032** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic

phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 1034 as illustrated in Figure 20: The substrate was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted to give the product.

Synthesis of compound 1038 as illustrated in Figure 20: Synthesis was carried out via a Mitsunobu inversion using identical conditions as published in Saiah et al. *Tet. Lett.* **1992**, *33*, 4317; Johnson et al *J. Am. Chem. Soc.* **1964**, *86*, 118. The reaction mixture is then worked up by dilution with ethyl acetate (20 mL) and is added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compound 58 as illustrat d in Figure 20: To the CBZ-protected amine (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product.

Synthesis of compound 59 as illustrated in Figure 20: CBZ-protected amine **1038** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product. Next, to an ice-cooled solution of substrate (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture was allowed to stir for 20 min., and then benzyl bromide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford product. The mother liquor is then chromatographed to give the product to give **58**.

Synthesis of compound 60 as illustrated in Figure 20:

To an ice-cooled solution of substrate **1038** (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture was allowed to stir for 20 min., and then methyl iodide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford product. The mother liquor is then chromatographed to give the product to give intermediate product. Next, to the CBZ-protected amine (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product.

Synthesis of compound 64 as illustrated in Figure 20: To the CBZ-protected amine **1034** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product.

Synthesis of compound 65 as illustrated in Figure 20:

CBZ-protected amine **1034** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product. Next, to an ice-cooled solution of substrate (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture was allowed to stir for 20 min., and then benzyl bromide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford product. The mother liquor is then chromatographed to give the product to give **65**.

Synthesis of compound 66 as illustrated in Figure 20: To an ice-cooled solution of substrate **1034** (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture was allowed to stir for 20 min., and then methyl iodide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and

concentrated to afford product. The mother liquor is then chromatographed to give the product to give intermediate product. Next, to the CBZ-protected amine (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product.

Synthesis of compound 1040 as illustrated in Figure 21: Piperidine compound **1039** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (0.10 Molar) at 0 °C under argon was added CBZ-Cl (1.1 equivalents; Aldrich) and the mixture was stirred at 0 °C for 20 min. and then quenched with a few drops of water. Saturated ammonium chloride solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then neutralized with saturated sodium bicarbonate solution, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product.

Synthesis of compound 1041 as illustrated in Figure 21:

To a solution of substrate (0.5 mmol) in anhydrous DMF (5 mL) was added TBDPSCl (1.05eq: Aldrich), Et_3N (1.1 eq), and a catalytic amount of imidazole. The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H_2O (30 mL). The organic layer was then washed with

sat'd $NH_4Cl_{(aq.)}$ solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield product. The mother liquor is then chromatographed to give the product **1041**.

Synthesis of compound 1042 as illustrated in Figure 21: To an ice-cooled solution of substrate (0.2 mmol) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (2.2 eq.). The mixture was allowed to stir for 20 min., and then methyl iodide or benzyl bromide (1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford product. The mother liquor is then chromatographed to give the product **1042**.

Synthesis of compound 1043 as illustrated in Figure 21: The substrate was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted to give the product.

Synthesis of compound 1044 as illustrated in Figure 21: Compound **1043** was disolved in 0.10 Molar solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1

equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product **1044**.

Synthesis of compound 1045 as illustrated in Figure 21: The substrate **1044** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N $HCl_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product.

Synthesis of compounds 86 or 87 as illustrated in Figure 21: CBZ-protected amine **1045** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the

suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product compound **86 or 87**, depending on whether benzyl or methyl.

Synthesis of compounds 1046 as illustrated in Figure 22: Piperidine compound **1041** (290 mg, 1.09 mmol; Aldrich) in methylene chloride (0.10 Molar) at 0 °C under argon was added acetic anhydride (3.3 equivalents; Aldrich), triethyl amine (3.3 equivalents) and the mixture was stirred at 0 °C for 20 min. and then quenched with a few drops of water. Saturated ammonium chloride solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then neutralized with saturated sodium bicarbonate solution, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. The mother liquor is then chromatographed to give the product.

Synthesis of compound 1047 as illustrated in Figure 22: The substrate was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted to give the product.

Synthesis of compound 1048 as illustrated in Figure 22: Compound **1047** was disolved in 0.10 Molar

solution of acetone and then 1.5 M sulfuric acid was added at 0 °C. Next chromium (VI) oxide (1.1 equivalents; Aldrich) was added and the mixture was allowed to stir for 2 hours. The reaction is worked up by adding EtOAc and washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. The mother liquor is then chromatographed to give the product.

Synthesis of compound 1049 as illustrated in Figure 22: The substrate **1048** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N $HCl_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product. Compound is then redissolved in dry methanol (3 mL) and sodium methoxide (0.30 equivalents added and the mixture is stirred for 18 hours at reflux. The is concentrated *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the

desired product **1049**.

Synthesis of compounds 88 as illustrated in Figure 22: CBZ-protected amine **1049** (0.092 mmol) was dissolved in MeOH (2 mL) and 20% palladium hydroxide on carbon (10 mg) was added and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min., TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield product compound **88**.

Synthesis of Compound 1050A as illustrated in Figure 27.

Step 1) To a solution of commerically available trans-3-hydroxyproline is added 1.1 equivalents BOC-ON (Aldrich) in a 1:1 v/v solution water/ dioxanes and NaOH (1N solution) and stirred at 0 °C for 12 hours. The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired protected product.

Step 2) As illustrated in figure 27 the substrate (70 mg, 0.213 mmol; vida supra), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl

acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired product **1050A**.

Synthesis of Compound 1050B as illustrated in Figure 27.

Step 1) To an ice-cooled solution of N-(benzyloxycarbonyl)-cis-3'-hydroxyproline (290 mg, 1.09 mmol; vida supra) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (96 mg, 2.40 mmol, 2.2 eq.). The mixture was allowed to stir for 20 min., and then benzyl bromide (0.14 mL, 1.20 mmol, 1.1 eq.) was added. The mixture was allowed to stir for 1 hr at 0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. (205 mg, 53%) The crude material was carried on without purification.

Step 2) To the crude yellow oil (0.982 mmol) was added TBAF (1M in THF) (1.9 mL, 1.9 mmol, 2 eq.), and the solution was stirred 2 hr at ambient temperature. The solution was concentrated in vacuo and

immediately applied to a short silica gel column, eluting with 33% ethyl acetate in hexanes. The product was isolated as a white foam (443 mg, 95%).

Synthesis of Compound 1050 as illustrated in Figure 27.

As illustrated in figure 13, steps i-ii, the substrate **11** (70 mg, 0.213 mmol), is dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropylethylamine, (122 µl, 0.703 mmol) are added and the mixture is stirred for 30 minutes at room temperature. The secondary amine **1050B** (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product which is directly carried on to the next step for oxidation of the secondary alcohol as follows. The secondary alcohol (21 mg, 0.044 mmol) is dissolved in dry CH_2Cl_2 (2 mL), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture is stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 mL) and

quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 mL) and sodium thiosulfate. The aqueous phase is extracted with ethyl acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gives the respective product **1050** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%) as a colorless oil.

Synthesis of *N*-benzyl-*cis*-and *trans*-2,5-dicarbmethoxy-pyrrolidines Compound 1051A (Figure 28): *N*-benzyl-*cis*-and *trans*-2,5-dicarbmethoxypyrrolidine were synthesized according to the procedure of Cignarella and Nathansohn (Cignarella, G. Nathansohn, G., *JOC* , **1961**, *26*, 1500.) The two diastereomers (*cis* and *trans*) were separated by flash chromatography (1:9 EA/H to 1:4 EA/H). Analytical data matched that reported by Kemp and Curran (Kemp, D.S., Curran, T.P. *JOC*, **1988**, 53, 5729)

Synthesis of Cis-(2S,5R)-dicarbmethoxypyrollidine (Step 1, intermediate to compound 1051B (figure 28)): N-benzyl-cis-(2S, 5R)-dicarbmethoxypyrrolidine (1.0 equivalents) was dissolved in MeOH (0.10 M) and a catalytic amount of 10% palladium on carbon was added. The mixture was stirred vigorously under a balloon of hydrogen until TLC indicated that the reaction was complete. Filtration and concentration afforded a clean product.

Synthesis of N-(benzyloxycarbonyl)-dimethyl pyrrolidine-(2S,5R)-dicarboxylate (Step 2, intermediate to compound 1051B (figure 28)): To the crude substrate dimethyl pyrrolidine-2,5-dicarboxylate (869 mg, 4.06 mmol) was added water (20 mL). The solution was cooled to 0 °C in an ice bath, and 0.3 M K_2CO_3 was added dropwise until pH = 9. CbzCl (1.1 eq, 4.5 mmol) was added and the mixture was stirred 30 min at 0°C and 30 min at ambient temperature. The aqueous layer was extracted with EtOAc (3 x 20 mL) and was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield a yellow oil. Purification by flash chromatography (1:3 EA/ H) yielded a clear oil. (1.2 g) Rf (1:1 EA/ H) = 0.48.

Synthesis of N-(benzyloxycarbonyl)-cis-(2S,5R)-dimethanol pyrrolidine compound 1051B: The substrate N-(benzyloxycarbonyl)-dimethyl pyrrolidine-(2S, 5R)-dicarboxylate (766 mg, 2.3 mmol) was dissolved in anhydrous THF (15 mL) and the solution was cooled to -78 °C in a dry ice/acetone bath. To this solution was added DIBAL-H (1.5 M in toluene) (14.2 mL, 9.6 mmol, 4.2 eq.). The solution was allowed to stir at -78°C, and gradually warmed to 0 °C during the course of 1 hr. The reaction was then quenched with 1N HCl (a few drops), and THF removed in vacuo. The slurry was extracted with EtOAc (3 x 30 mL) and washed with brine (30 mL). After drying over $MgSO_4$ and concentratedin vacuo to yield a yellow oil. Flash column purification in 1:1 EA/ H gave the desired diol.

Synthesis of N-(benzyloxycarbonyl)-cis-(2S)-methanol-(5R)-(butyroxymethyl) pyrrolidine (Step 1, intermediate to compound 1051C (figure 28)): The substrate diol (800 mg, 3.02 mmol) was dissolved in vinyl butyrate (10 mL), and 553 mg of Lipase AK was added. The reaction was stirred slowly at ambient temperature until TLC indicated that starting material was gone. (60 hr.). The reaction mixture was diluted with CH2Cl2, filtered through Celite, and concentrated. After flash chromatography eluting with a gradient of 1:4 to 1:1 ethyl acetate in hexanes, the product was isolated as a colorless oil. (675 mg, 67% yield, 85% ee) (The enantiomeric excess was determined by conversion to the Mosher ester followed by 19F NMR analysis).

Synthesis of N-(benzyloxycarbonyl)-5-cis-(butyroxymethyl)-L-proline compound 1051C (figure 28). To an ice cooled solution of CrO_3 (6.83 mmol, 3.75 eq.) in 1.5M H_2SO_4 (aq.) (9 mL) was added substrate (611 mg, 1.82 mmol) in acetone (30 mL + 10 mL wash). The resulting orange solution was stirred vigorously at 0°C for 30 min., and then at ambient temperature for 7 hrs. Ethyl ether (40 mL) was then added and the organic layer was washed with brine solution (20 mL), dried over MgSO4, and concentrated in vacuo to afford crude product. This product which was subjected to a short column of silica eluting with 100% ethyl acetate to give pure product as a colorless oil (571 mg, 89%) ^{1}H NMR (CD_3OD, 250 MHz) (peaks broadened by rotamers) δ = 7.45-7.20 (5H, m), 5.25-4.90 (3H, m), 4.40-4.25 (1H, m), 4.25-4.00 (3H, m), 2.38-2.15 (2H, m), 2.15-1.73 (4H, m), 1.72-1.42

(2H, m), 1.00-0.78 (3H, m); ^{13}C NMR (CD_3OD) d = 175.7, 174.9, 156.4, 156.0, 137.6, 129.5, 129.3, 129.2, 129.0, 128.8, 128.5, 128.4, 68.3, 68.1, 65.4, 65.0, 61.3, 60.9, 58.6, 58.1, 36.7, 29.9, 28.9, 28.1, 19.2, 13.9; FABHRMS (NBA) m/e 350.1593 ([M + H]+ C18H23NO6 requires 350.1604.

Synthesis of N-(Benzyloxycarbonyl)-5-cis-(butyroxymethyl)-L-proline-tert-butyl amide compound 1051D (figure 28): The substrate i (13.9 mmol) was dissolved in dry CH_2Cl_2 (20 mL). HOBT, 1-hydroxybenzotriazole hydrate (2.07 g, 15.3 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 mL, 15.3 mmol), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was diluted with ethyl acetate (100 mL), and washed with water (2 x 20 mL), 1 N HCl $_{(aq.)}$ (10 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (10 mL), water (10 mL), brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave the title compound as a colorless oil.

Synthesis of N-(Benzyloxycarbonyl)-5-cis-hydroxymethyl-L-proline-tert-butyl amide compound 1052A (intermediate en route to compound 1051E (figures 28 and 29): The substrate was dissolved in MeOH: H_2O (8 mL: 4 mL) and cooled to 0°C. LiOH was added (128 mg, 5.0 eq.) and the mixture stirred for 20 min. at 0°C. The mixture was neutralized with 1N HCl and then extracted into EtOAc, dried over MgSO4,

and filtered to afford product which was used in the next step without purification.

Synthesis of 5-cis-hydroxymethyl-L-proline-tert-butyl amide (compound 1051E (figure 28): Intermediate compound **1052A** (1.0 equivalents; vida supra) was dissolved in MeOH (0.10 M) and a catalytic amount of 10% palladium on carbon was added. The mixture was stirred vigorously under a balloon of hydrogen until TLC indicated that the reaction was complete. Filtration and concentration afforded a clean product.

Synthesis of compound 1051 (figure 28) Using the general procedure as follows: To the pyrrolidine derivative **1051 E** (20 mg, 0.091 mmol) was added dry methanol (2 mL), Cbz-phenylalanyl epoxide **21** (27 mg, 0.091 mmol, 1.0 eq; vida supra) and triethylamine (14 µL, 0.100mmol, 1.1eq.). The solution was refluxed for 32 h, and then concentrated *in vacuo*. Flash chromatography, eluting with ethyl acetate provides the desired product as a clear oil.

Synthesis of N-(Benzyloxycarbonyl)-5-cis-(tert-butyl-dimethyl-silyl-oxymethyl)-L-proline-tert-butyl amide (intermediate to compound 1052B; (figure 29) A solution of N-(benzyloxycarbonyl)-5-cis-hydroxymethyl-L-proline-tert-butyl amide **1052A** (81 mg, 0.24 mmol; vida supra) in CH2Cl2 (3 mL) was cooled to 0°C under an argon bed. 2,6-lutidine (0.48 mL, 56 mL, 2.0eq.) and tert-butyldimethylsilyltriflate (88 mL, 0.38 mmol, 1.6 eq.) were added subsequently. After stirring for 1

hr at 0°C, the reaction was quenched by pouring the reaction mixture into an ice cold mixture of sat'd $NaHCO_3$(aq)solution: H_2O (5 mL: 25 mL) and extracting into CH_2Cl_2 (3 x 20 mL) The pooled organic layers were washed with 10% $CuSO_4$(aq) solution (2 x 20mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography eluting with 25% ethyl acetate in hexanes gave the intermediate compound as a colorless oil (85mg, 79%). Rf = 0.58 (1:1 EtOAc/ Hexanes). 1H NMR ($CDCl_3$, 250 MHz) (peaks broadened by rotamers) d = 7.33-7.26 (5H, m), 5.14 (2H, br s), 4.18-4.12 (1H, m), 4.08-3.90 (1H, m), 3.83-3.71 (2H, m), 2.39-2.00 (2H, m), 2.00-1.82 (2H, m), 1.24 (9H, s), 0.86 (9H, s), 0.03 (6H, br s); 13C NMR ($CDCl_3$, 62.5 MHz) 136.1, 128.4, 128.1, 127.9, 67.2, 64.3, 50.7, 28.4, 25.9; FABHRMS (NBA) m/e 581.1824 ([M + Cs]+ $C_{24}H_{40}N_2O_4Si$ requires 581.1822. Intermediate compound (1.0 equivalents; vida supra) was dissolved in MeOH (0.10 M) and a catalytic amount of 10% palladium on carbon was added. The mixture was stirred vigorously under a balloon of hydrogen until TLC indicated that the reaction was complete. Filtration and concentration afforded a clean product to give **1052B**.

Synthesis of 2S,3S)-3-(N-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-5'-cis-(tert-butyldimethylsilyloxymethyl)-L-prolyl-tert-butyl amide (intermediate en route to compound 1052; figure 29, step 1) α-Hydroxy acid 11 (62.5 mg, 0.19 mmol, 1.0 eq; vida supra) , EDC (40 mg, 1.1 eq.), HOBT (28.2 mg, 1.1 eq.) were stirred in dry DMF (1 mL) at

ambient temperature, under argon. After preactivation for 30 min., the amine **1052B** (59 mg, 1.0 eq) dissolved in dry DMF (0.5 mL + 2 x 0.5 mL washes) was cannulated into the activated acid mixture. After stirring for 12 hr, the reaction mixture was concentrated in vacuo, and EtOAc was added. The organic layer was washed with water (2 x 5 mL), dried over MgSO4, and solvent removed under reduced pressure. Flash chromatography eluting with 20% ethyl acetate in hexanes gave intemediate as a white crystalline solid (77mg, 65%). Rf= 0.59 (1:2 EtOAc/ Hexanes); 1H NMR (CDCl3, 400MHz): (mixture of two rotamers) d = 7.32-7.09 (20H, m), 6.86 (1H, s), 6.42 (1H, s), 5.63 (1H, d, J= 8.9 Hz), 5.42 (1H, d, J= 8.8 Hz), 5.07 (1H, d, J=12.0 Hz), 5.00 (1H, d, J= 12.0 Hz), 5.08-4.92 (2H, m), 4.53-4.38 (2H, m), 4.37-4.19 (3H, m), 4.12-3.97 (3H, m), 3.89-3.71 (2H, m), 3.63-3.52 (1H, m), 3.52-3.47 (1H, m), 2.90-2.62 (4H, m), 2.50-2.32 (1H, m), 2.20-1.60 (9H, m), 1.30 (9H, s), 1.27 (9H, s), 0.87 (9H, s), 0.86 (9H, s), 0.08 (3H, s), 0.06 (3H, s), 0.04 (3H, s), 0.01 (3H, s); 13C NMR (CDCl3, 100MHz); FABHRMS (NBA) m/e 758.2618([M + Cs]+ C34H51N3O6Si requires 758.2601.

Synthesis of intermediate to compound 1052 (step 2, figure 29) General Dess-Martin Oxidation Procedure:(2S,3S)-3-(N-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-5'-cis-(tert-butyldimethylsilyloxymethyl)-L-prolyl-tert-butyl amide

The substrate X (28 mg, 0.044 mmol) was dissolved in dry CH2Cl2 (2 mL) and Dess-Martin periodinane (26 mg, 0.088 mmol, 2.0 eq.) was added. The reaction mixture

was stirred at ambient temperature for 24 hr, then diluted with ethyl acetate (10 mL), and quenched by addition of sat'd sodium bicarbonate(aq.)(5 mL), and sodium thiosulfate. The aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over $MgSO_4$, and concentrated in vacuo to give the crude product. Flash chromatography eluting with 50% ethyl acetate in hexanes gave the desired product as a white solid (1:1 mixture of diastereomers). (26 mg, 95%) Rf = 0.47 (1:2 EtOAc/ Hexanes); 1H NMR ($CDCl_3$, 400MHz); d = 7.36-7.13 (20 H, m), 6.40 (1H, s), 6.07 (1H, s), 5.36 (1H, d, J= 7.2 Hz), 5.18 (1H, d, J= 6.8 Hz), 5.11-4.97 (5h, m), 4.96-4.90 (1H, m), 4.50-4.47 (1H, m), 4.22-4.18 (2H, m), 3.99 (1H, dd, J= 9.6, 4.9 Hz), 3.96-3.87 (1H, m), 3.75 (1H, dd, J= 10.0, 4.8 Hz), 3.53-3.32 (3H, m), 3.27 (1H, dd, J= 14.2, 5.5 Hz), 3.18 (1H, dd, J= 14.1, 8.1 Hz), 2.91 (1H, dd, J= 14.0, 9.7 Hz), 2.40-2.28 (1H, m), 2.25-2.16 (1H, m), 2.08-1.97 (1H, m), 1.96-1.78 (2H, m), 1.72-1.52 (3H, m), 1.34 (9H, s), 1.31 (9H, s), 0.88 (9H, s), 0.86 (9H, s), 0.09 (3H, s), 0.06 (3H, s), 0.04 (3H, s), 0.03 (3H, s); 13C ($CDCl_3$, 100MHz); IR (NaCl) vmax 3332.3, 2955.0, 2856.0, 2358.1, 1714.7, 1634.1, 1537.8, 1454.8, 1258.0, 1094.6; FABHRMS (NBA) m/e 756.2469 ([M + Cs]+ $C_{34}H_{49}N_3O_6Si$ requires 756.2445.

Synthesis of compound 1052 (as illustrated in figure 29): To a 1.0 Molar solution of 1.0 equivalents substrate, TBAF (1.0 M solution in THF) was added (0.34 mL, 1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced

pressure. The crude material was applied to a short column of silica gel and eluted with 33% hexanes in ethyl acetate to give a yellow oil (162 mg, 94%)

Synthesis of N-(Benzyloxycarbonyl)-5-cis-(methoxymethyl)-L-proline-tert-butyl amide compound 1053A (figure 29). A solution of **1052A** (156 mg, 0.467 mmol) in anhydrous DMF (3 mL) was cooled to 0°C under argon. NaH, 60% disp. in mineral oil, (28.0 mg, 0.70 mmol, 1.5eq.) was added and the suspension was allowed to stir for 20 min. Iodomethane (116 mL, 1.8 mmol, 4.0 eq.) was then added and the mixture stirred for 3 hr at 0°C. The reaction was quenched by addition of a few drops of 1N HCl(aq.) solution, and then diluted with H2O (10 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL), dried over MgSO4, filtered, and evaporated under reduced pressure. Flash chromatography eluting with 50% ethyl acetate in hexanes gave an intermediate as a colorless oil (146 mg, 89%). Rf = 0.25 (1:1 EtOAc/ Hexanes). 1H NMR (CDCl3) 13C NMR (CDCl3, MHz) FABHRMS (NBA) m/e 349.2119 ([M+ H]+ C19H28N2O4 requires 349.21270

To a solution of above intermediate (85 mg, 0.19 mmol) in MeOH (3 mL) was added a catalytic amount of 10% Pd/C and the mixture stirred under a balloon of H2 at ambient temperature. After 20 min, TLC indicated that the reaction was complete and the mixture was filtered through a bed of Celite and washed with MeOH. Removal of solvent under reduced pressure gave a colorless oil (89mg, quant.) as compound **1053A** which was used in the next step without further purification. 1H NMR (CDCl3, 400MHz)

d = 5.64 (1H, br s), 4.21 (1H, dd, J= 9.2, 5.0 Hz), 3.78-3.68 (1H, m), 3.53 (1H, dd, 9.8, 4.1Hz), 3.46-3.37 (1H, m), 3.42 (3H, s), 2.41-2.31 (m, 1H), 2.06-1.89 (2H, m), 1.69-1.60 (1H, m), 1.36 (9H, s). 13C (CDCl3, 100 MHz) d 171.2, 73.4, 60.2, 59.2, 59.0, 51.0, 30.7, 28.6, 27.0. FABHRMS (NBA) m/e 215.1764 ([M + H]+ C11H22N2O2 requires 215.1760.

Synthesis of (2S,3S)-3-(N-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-5'-cis-(methoxymethyl)-L-prolyl-tert-butyl amide (intermediate to compound 1053, figure 29)

Synthesized according to the General Coupling Procedure outlined above and as illustrated in synthesis of compound **1052** (figure 29). Rf= 0.30 (EtOAc: Hexanes 1:2) 1H NMR (CDCl3, 400 MHz): (mixture of rotamers) d = 7.33-7.17 (20 H, m), 6.91 (2H, d, J= 5.6 Hz), 5.75 (1H, d, J= 8.8 Hz), 5.64 (1H, d, J= 8.8 Hz), 5.07 (1H, d, J=12.3 Hz), 5.03 (2H, s), 4.91 (1H, d), 4.61-4.55 (1H, m), 4.53-4.38 (3H, m), 4.28-4.15 (4H, m), 4.02-3.95 (1H, m), 3.93-3.86 (2H, m), 3.81-3.75 (1H, m), 3.46 (1H, d, J= 8.7 Hz), 3.39-3.26 (3H, m), 3.35 (3H, s), 3.20 (3H, s), 2.93-2.84 (3H, m), 2.80-2.73 (1H, m), 2.42-2.35 (1H, m), 2.15-1.82 (3H, m), 1.80-1.74 (1H, m), 1.72-1.62 (1H, m), 1.30 (9H, s), 1.27 (9H, s); 13C NMR (CDCl3, 100MHz) d =172.4, 171.6, 171.1, 169.9, 156.2, 156.0, 137.6, 137.2, 136.4, 136.2, 129.5, 129.4, 129.1, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 126.9, 126.4, 73.2, 71.2, 71.0, 70.2, 66.6, 66.4, 61.8, 60.8, 60.7, 60.4, 59.5, 59.0, 58.8, 58.7 58.0, 55.3, 54.7, 54.5, 51.1, 50.8, 35.1, 29.8, 29.6, 28.5, 27.9, 26.2, 24.1, 21.0, 14.1; FABHRMS (NBA) m/e

658.1879 ([M + Cs]+ C29H39N3O6 requires 658.1893.

Synthesis of (2S,3S)-3-(N-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-5'-cis-(methoxymethyl)-L-prolyl-tert-butyl amide (compound 1053; figure 29)

Synthesized according to the General Dess-Martin procedure as noted above and as illustrated in the example for compound **1052 (figure 29)**. Rf = 0.40 (1:1 EtOAc/ Hexanes); 1H NMR (CDCl3, 400 MHz) (1:1 mixture of diastereomers) d = 7.33-7.16 (20H, m), 6.88 (1H, s), 6.79 (1H, s), 5.70 (1H, d, J = 8.6 Hz), 5.26 (1H, d, J = 7.1 Hz), 5.18-5.12 (2H, m), 5.08-4.92 (4H, m), 4.48-4.43 (2H, m), 4.28-4.22 (1H, m), 4.14-4.10 (1H, m), 4.09-4.02 (1H, m), 3.45 (1H, dd, J = 9.5, 1.9 Hz), 3.37 (3H, s), 3.36-3.17 (6H, m), 3.15 (3H, s), 2.94 (1H, dd, J = 13.9, 9.4 Hz), 2.42-2.30 (1H, m), 2.20-2.10 (1H, m), 2.08-1.82 (5H, m), 1.81-1.72 (1H, m), 1.34 (9H, s), 1.30 (9H, s); 13C (CDCl3, 100MHz); 198.0, 197.0, 171.1, 169.4, 164.9, 163.9, 155.9, 155.6, 136.3, 136.1, 129.7, 129.3, 128.7, 128.5, 128.4, 128.3, 128.1, 127.0, 126.8, 74.3, 70.4, 67.1, 66.8, 63.6, 61.2, 59.8, 58.8, 58.6, 58.5, 57.9, 57.6, 51.0, 50.9, 37.2, 29.9, 28.6, 27.4, 25.7, 24.9; IR (NaCl) vmax 3331.1, 2966.8, 2340.9, 1717.4, 1646.2, 1540.6, 1455.8, 1252.9, 1111.7, 1047.1; FABHRMS (NBA) m/e 656.1714 ([M + Cs]+ C29H37N3O6 requires 656.1737; (Found C, 66.24; H, 7.21; N, 7.78; C29H37N3O6 requires C, 66.50, H, 7.13; N, 8.03).

5.

Synthesis of *para*-(*ortho*-nitrobenzyloxy)-hydroxybenzyl bromide compound 1054A as illustrated

in figure 30

Step 1) To a solution of para-hydroxybenzyl alcohol (2.0 g, 16.1 mmol; Aldrich) dissolved in dry acetone (15 mL) was added K2CO3 (4.45 g, 32.2 mmol, 2.0 eq.) and ortho-nitrobenzylbromide (3.83 g, 17.7 mmol, 1.1 eq). The mixture was stirred at ambient temperature for 16 hr and then the solution was concentrated in vacuo. The residue was taken up in EtOAc (100 mL) and then the organic layer was washed with H2O (2 x 30 mL), brine (30 mL) and dried over MgSO4. The product was recrystallized from Et2O/hexanes to give yellow needles (2.0 g, 60%) of para-(ortho-nitrobenzyl)-hydroxybenzyl alcohol. 1H NMR (CDCl3, 400 MHz) d = 8.16 (1H, dd, J = 8.2, 1.3 Hz), 7.88 (1H, dd, 7.9, 1.0 Hz), 7.68 (1H, dt, J = 7.5, 1.2 Hz), 7.51-7.46 (1H, m), 7.32-7.26 (2H, m), 6.98-6.95 (2H, m), 5.48 (2H, s), 4.61 (2H, s); 13C NMR (CDCl3, 100 MHz) d = 157.6, 133.9, 128.7, 128.5, 128.2, 124.9, 114.8, 66.8, 64.9;

Step 2) To a suspension of Ph_3P (1.03 g, 3.94 mmol, 1.02 eq.) in CH_3CN (10 mL) cooled to 0°C. Bromine (0.20 mL, 3.86 mmol, 1.0 eq.) was added dropwise. The ice bath was then removed and a solution of para-(ortho-nitrobenzyl)hydroxybenzyl alcohol in 5mL of CH3CN was added via cannulation. After stirring for 10 min., the CH3CN was removed in vacuo and the residue was extracted with hexanes (5 x 30mL) and filtered through a pad of Celite. Concentration of the hexanes in vacuo yielded a fluffy white solid compound **1054A** (1.1g, 88%); 1H NMR (CDCl3, 400 MHz) d = 8.17 (1H, d, J = 8.2 Hz), 7.86 (1H, d, J = 7.8 Hz), 7.68 (1H, t, J = 7.4 Hz), 7.54-7.49 (1H, m), 7.34 (2H, d, J = 8.4 Hz), 6.94 (2H, d, J = 8.4 Hz), 5.49

(2H, s), 4.50 (2H, s);

Synthesis of trans-3'-(tert-butyldimethylsilyloxy) L-proline tert-butyl amide compound 1054C as illustrated in Figure 30

To a solution of N-(benzyloxycarbonyl)-trans-3'-hydroxyproline tert-butyl amide **1054B** (739 mg, 2.31 mmol; vida supra; synthesized as **1050A, figure 27**) in anhydrous DMF (10mL) was added TBDMSCl (366 mg, 2.42 mmol, 1.05eq), Et_3N (0.35 mL, 2.54 mmol, 1.1 eq), and a catalytic amount of DMAP. The solution was stirred at ambient temperature for 15 hr and then was partitioned between EtOAc (60 mL) and H2O (30 mL). The organic layer was then washed with sat'd NH4Cl(aq.) solution (2 x 30 mL), water (30 mL), brine (30 mL), dried over MgSO4, and concentrated in vacuo to yield the crude material as a yellow oil. The yellow oil was then dissolved in MeOH (20 mL) and a catalytic amount of 10% Pd/C was added. The mixture was stirred vigorously under a balloon of H_2 for 1 hr. The mixture was then filtered through Celite and concentrated in vacuo to yield a yellow oil (690 mg, 99 %); 1H NMR (CDCl3, 400 MHz) δ = 6.97 (1H, s), 4.46 (1H, dd, J = 9.1, 4.6 Hz), 3.76 (1H, d, J = 4.04 Hz), 3.42 (1H, dt, J = 10.6, 7.6 Hz), 3.21 (1H, ddd, J = 10.6, 7.5, 5.8 Hz), 1.34 (9H, s), 0.89 (9H, s), 0.12 (3H, s), 0.11 (3H, s); 13C NMR (CDCl3, 100 MHz) d = 168.3, 75.5, 67.8, 51.4, 44.1, 33.8, 28.6, 25.7, -4.6, -4.8; FABHRMS (NBA) m/e 301.2317 ([M + H]+ C15H32N2O2Si requires 301.2311);

Synthesis of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-hydroxy-4-ph nylbutyryl-trans-3'-(tert-

butyldimethylsilyloxy)-L-prolyl-tert-butyl amide compound 1054D as illustrated in figure 30. Synthesized according to the general peptide coupling procedure outlined above as exemplified for compound **1052, figure 29.** ^{1}H NMR (CDCl3, 400 MHz) d = 7.28-7.08 (10H, m), 6.48 (1H, s), 5.57 (1H, d, J = 8.8 Hz), 5.08-4.93 (2H, m), 4.63-4.58 (1H, m), 4.52-4.38 (1H, m), 4.26 (1H, br s), 4.20-3.61 (4H, m), 1.24 (9H, s), 0.88 (9H, s), 0.09 (6H, s); 13C NMR (CDCl3, 100 MHz) d = 171.4, 170.0, 168.5, 156.6, 156.1, 137.4, 136.2, 135.9, 129.1, 128.8, 128.4, 128.0, 127.9, 127.4, 126.4, 73.1, 71.6, 71.2, 69.7, 69.2, 66.6, 66.5, 54.8, 54.5, 51.4, 51.3, 45.9, 45.5, 34.5, 33.6, 32.9, 31.8, 28.5, 28.4, 25.7, 17.9, 14.1;

Synthesis of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-acetoxy-4-phenylbutyryl-trans-3'-(tert-butyldimethylsilyloxy)-L-prolyl-tert-butyl amide intermediate compound to 1054E as illustrated in figure 30. To a solution of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-hydroxy-4-phenylbutyryl-trans-3'-(tert-butyldimethylsilyloxy)-L-prolyl-tert-butyl amide **1054D** (216 mg, 0.35 mmol) in anhydrous CH_2Cl_2 was added acetic anhydride (67 mL, 0.71 mmol, 2.0 eq.), pyridine (71 mL, 0.882 mmol, 2.5 eq.). It was difficult to tell whether the reaction had gone to completion by monitoring by TLC, since the Rf values of the starting material and product were virtually identical. After stirring the reaction for 15 hr under argon, EtOAc (40 mL) was added and the solution was washed with sat'd NH4Cl(aq.) (20 mL), sat'd NaCO3 (aq.) (20 mL), water (20 mL) and brine (20 mL). The organic layer was dried over MgSO4,

filtered, and concentrated to yield a white fluffy solid. (211 mg, 92%); 1H NMR (CDCl3, 400 MHz) δ = 7.33- 7.13 (10H, m), 6.47 (1H, s), 5.57 (1H, d), 5.36-5.30 (1H, m), 5.02 (2H, s), 4.69-4.58 (1H, m), 4.39-4.31 (1H, m), 4.20-4.09 (1H, m), 3.87-3.57 (2H, m), 2.98-2.79 (2H, m), 2.26-2.22 (1H, m), 2.08 (3H, s), 1.95-1.85 (1H, m), 1.31 (9H, s), 0.87 (9H, s), 0.099 (6H, s); 13C NMR (CDCl3, 100 MHz) d = 170.2, 169.9, 168.6, 167.8, 167.4, 166.5, 155.9, 136.9, 136.1, 128.9, 128.5, 128.4, 128.0, 127.8, 127.7, 126.6, 76.5, 73.3, 72.8, 71.3, 69.5, 69.4, 66.7, 66.5 52.7, 52.4, 51.4, 51.1, 45.5, 45.2, 35.5, 34.3, 33.8, 31.0, 28.5, 28.2, 25.6, 25.5,20.5, 20.3, 17.9, 17.6; FABHRMS (NBA) m/e 786.2571 ([M + Cs]+ C35H51N3O7Si requires 786.2551);

Synthesis of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-acetoxy-4-phenylbutyryl-trans-3'-hydroxy-L-prolyl-tert-butyl amide compound 1054E as illustrated in figure 30. The substrate (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-acetoxy-4-phenylbutyryl-trans-3'-(tert-butyldimethylsilyloxy)-L-prolyl-tert-butyl amide (1.0 equivalents; vida supra) was dissolved in THF and cooled to 0°C. TBAF (1.0 M solution in THF) was added (0.34 mL, 1.05 eq.) and the reaction monitored by TLC. After 30 min. at 0°C, the reaction was complete and the solvent was evaporated under reduced pressure. The crude material was applied to a short column of silica gel and eluted with 33% hexanes in ethyl acetate to give a yellow oil (162 mg, 94%)

Synthesis of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-hydroxy-4-phenylbutyryl-trans-3'-(para-(ortho-nitrobenzyl)benzyloxy)-L-prolyl-tert-butyl amide compound 1054F, figure 30.

To a solution of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-acetoxy-4-phenylbutyryl-trans-3'-hydroxy-L-prolyl-tert-butyl amide dissolved in anhydrous THF (3 mL) was added para-(ortho-nitrobenzyl)hydroxybenzylbromide (106.4 mg, 0.331 mmol), 4A molecular sieves (spatula-full), and silver triflate (a spatula-full). The reaction was complete in 10 minutes at ambient temperature so it was immediately filtered through a pad of Celite and washed with Et2O. After flash chromatography eluting with 50% ethyl acetate in hexanes, a colorless oil was isolated (165 mg, 70%).

The substrate (2S,3S)-3-(N-Benzyloxycarbonyl)amino-2-acetoxy-4-phenylbutyryl-3'-trans-[para-(ortho-nitrobenzyl)benzyloxy]-L-prolyl-tert-butyl amide was dissolved in MeOH: H2O (7 mL: 1 mL) and cooled to 0°C. K_2CO_3 (146 mg, 1.06 mmol, 5.0 eq.) was added and the cloudy mixture was allowed to warm gradually to RT. Monitoring the reaction by TLC was difficult because the starting material and product had nearly identical Rf values. The reaction was stirred at RT for an additional 30 min., and then the MeOH was removed in vacuo. The resulting residue was partitioned between water (20 mL) and EtOAc (30 mL) and dried over MgSO4, filtered, and concentrated to yield a colorless oil (155 mg, 99%).

Synthesis of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-keto-4-phenylbutyryl-trans-3'-[para-

hydroxybenzyloxy]-L-prolyl-tert-butyl amide compound 1054. Step 1) Synthesized according to the general Dess-Martin oxidation procedure as outlined above for compound **1052, figure 29.**
Step 2) The substrate (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-keto-4-phenylbutyryl-trans-3'-[para-(ortho-nitrobenzyloxy)benzyloxy]-L-prolyl-tert-butyl amide was dissolved in CH_2Cl_2 (1 mL) and placed in the sunlight until TLC indicated that the reaction had gone to completion. The solution was concentrated in vacuo and purified by flash chromatography (eluent 33% ethyl acetate in hexanes) to yield a slightly yellow oil.

Alternative compounds 1059, 1060 and other hydroxybenzyloxyproline substituted α-keto amides The synthesis of the trans-3-meta-hydroxybenzyloxyproline substituted α-keto amides is done in an analogous fashion to that described above for the trans-3-para-hydroxybenzyl-substituted syste; **1059 and 1060** can be synthesized using the bromides shown in figure 30.

Synthesis of N-(benzyloxycarbonyl)-cis-4'-(benzyloxy)-L-proline succinate ester compound 1055A as illustrated in figure 31. Step 1) To an ice-cooled solution of N-(benzyloxycarbonyl)-cis-4'-hydroxyproline (290 mg, 1.09 mmol; Aldrich) in anhydrous DMF (2 mL) under argon was added NaH (60% disp. in mineral oil) (96 mg, 2.40 mmol, 2.2 eq.). The mixture was allowed to stir for 20 min., and then benzyl bromide (0.14 mL, 1.20 mmol, 1.1 eq.) was added. The mixture was allowed to stir for 1 hr at

0°C and then quenched with a few drops of water. Saturated $NaHCO_3$ solution was added (10 mL) and the aqueous layer was washed with ethyl ether. The aqueous layer was then acidified to pH 2 with 1N HCl, extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, and concentrated to afford a yellow oil. (205 mg, 53%) The crude material was carried on without purification.

Step 2) To a solution of N-(benzyloxycarbonyl)-cis-4'-(benzyloxy)-L-proline (41 mg, 0.115 mmol) in anhydrous CH_2Cl_2 (2 mL) was added EDC (68 mg, 0.356 mmol, 3.1 eq.) and N-hydroxysuccinimide (40 mg, 0.345 mmol, 3.0 eq.). The solution was stirred for 2 hrs. The solvent was removed, and the residue was partitioned between ethyl ether and water (10 mL/ 5 mL). The water layer was extracted with ethyl ether and the combined organic layers were washed with brine (10 mL) and dried over MgSO4. Concentration followed by flash chromatography eluting with 9% methanol in ethyl acetate yielded the desired product as a white solid (51 mg, 98%) ^1H NMR (CDCl3, 400MHz) (two distinct rotamers, only major rotamer noted here) d 7.38-7.25 (10H, m), 5.23 (1H, d, J = 12.4 Hz, ab-Cbz), 5.10 (1H, d, 12.4 Hz ab-Cbz), 4.75 (1H, dd, J = 9.4, 3.0 Hz, a-H), 4.63 (1H, d J = 12.5 Hz, ab-Bn), 4.48 (1H, d, J = 12.5 Hz, ab-Bn), 4.15-4.09 (1H, m, CH-OBn), 3.75-3.62 (2H, m, NCH2CHOBn), 2.82 (4H, s, succ.CH2), 2.65-2.62 (1H, m, NCH2CHOBnCH2), 2.47-2.34 (1H, m,NCH2CHOBnCH2);

Synthesis of N-(benzyloxycarbonyl)-cis--4'-(benzyloxy)-L-proline-isoleucine-glutamine-tert-butyl ester compound 1055B. To a solution of N-

(benzyloxycarbonyl)-cis-4'-(benzyloxy)-L-proline succinate ester **1055A** (28 mg, 0.0619 mmol) in anhydrous CH_2Cl_2 (2 mL) was added the dipeptide H_2N-Ile-Gln-(OtBu ester) (39 mg, 0.123 mmol, 2 eq.) and the solution was stirred at ambient temperature for 45 min. The solution was diluted with CH_2Cl_2 (5 mL) and then washed with sat'd NH_4Cl solution (5 mL), sat'd NaHCO3 solution, brine (5 mL) and dried over MgSO4. Concentration followed by flash chromatography with 8% methanol in ethyl acetate afforded the desired product as a white solid (40 mg, 99). 1H NMR (CDCl3, 400 MHz) d =7.36-7.27 (10H, m), 6.99 (1H, br s), 6.84 (1H, d, J = 8.6 Hz), 6.75 (1H, br s), 5.80 (1H, br s), 5.15 (2H, s), 4.53-4.32 (4H, m), 4.33-4.30 (1H, m), 4.12-4.09 (1H, m), 3.71 (1H, d, J = 11.9 Hz), 3.59-3.54 (1H, m), 2.64-2.61 (1H, m), 2.30-2.15 (4H, m), 2.00-1.85 (1H, m), 1.83-1.69 (1H, m), 1.53-1.38 (2H, m), 1.45 (9H, s), 1.00-0.93 (1H, m), 0.81-0.71 (6H, m); 13C NMR (CDCl3,100 MHz) d 175.2, 171.2, 170.5, 156.2, 137.4, 135.9, 81.9, 76.4, 70.5, 67.7, 60.6, 57.8, 53.4, 52.1, 36.7, 34.1, 31.7, 28.1, 24.5, 15.0, 11.2; FABHRMS (NBA) m/e 785.2520 ([M + Cs]+ C35H48N4O8 requires 785.2526);

Synthesis of cis-4-(benzyloxy)-L-proline-isoleucine-glutamine-tert-butyl ester compound 1055C as illustrated in figure 31.

To a solution of N-(benzyloxycarbonyl)-cis--4'-(benzyloxy)-L-proline-isoleucine-glutamine-tert-butyl ester **1055B** (60 mg, 0.092 mmol) in MeOH (2 mL) was added 20% palladium hydroxide on carbon (10 mg) and the suspension was stirred vigorously under a balloon of hydrogen at ambient temperature. After 5 min.,

TLC indicated that the reaction was complete. The suspension was filtered through a pad of celite and concentrated in vacuo to yield X (47 mg, quant.) 1H NMR (CD3OD, 400 MHz) d 7.33-7.23 (5H, m), 4.47 (1H, d, J=11.8 Hz), 4.41 (1H, d, J=11.8 Hz), 4.27-4.18 (2H, m), 4.17-4.13 (1H, m), 3.88 (1H, dd, J=7.8, 4.3 Hz), 3.20 (1H, d, J=11.7 Hz), 3.12 (1H, dd, J=11.7, 4.6 Hz), 2.36-2.17 (2H, m), 2.12-2.07 (1H, m), 1.92-1.75 (2H, m), 1.57-1.46 (1H, m), 1.49 (9, s), 1.16-1.05 (1H, m), 0.92-0.88 (3H, m), 0.85-0.75 (3H, m); 13C NMR (CD3OD, 100 MHz) d 177.4, 175.3, 173.3, 172.1, 139.6, 129.4, 128.9, 128.6, 82.9, 79.9, 71.9, 60.4, 59.0, 54.0, 53.0, 38.4, 37.1, 32.1, 32.5, 28.2, 25.8, 15.9, 11.5;

Synthesis of (2S,3S)-3-N-(benzyloxycarbonyl)-amino-2-keto-4-phenylbutyryl-cis-4'-benzyloxy-L-proline-isoleucine-glutamine-tert-butyl ester compound 1055 as illustrated in Figure 31. The following substances were combined in a flask, under a bed of argon: α-hydroxy acid **11** (26 mg, 0.080 mmol, 1.2 eq.), amine **1055C** (35 mg, 0.067 mmol), HBTU (30.5 mg, 0.080 mmol, 1.2 eq.), and HOBT (10.9 mg, 0.080 mmol, 1.2 eq.). Anhydrous THF (1 mL) was added, followed by DIEA (28 mL, 0.161 mmol, 2.4 eq.) and the solution was stirred for 18hr at ambient temperature. After removal of the solvent in vacuo, the resulting residue was taken up in EtOAc (10 mL) and washed with sat'd NH_4Cl(aq) (2 x 5 mL), sat'd $NaHCO_3$(aq)(2 x 5 mL), water (1 x 5 mL), and brine (1 x 5 mL). The organic layer was then dried over MgSO4, filtered, and concentrated in vacuo to yield a crude yellow oil. After subjecting the crude oil to flash

chromatography in 5% MeOH in CH_2Cl_2, a white solid was isolated (24 mg, 43% yield). Rf = 0.33 (9:1 CH_2Cl_2). 1H NMR(CDCl3, 400 MHz) (major rotamer only) d = 7.33-7.11 (15H, m), 7.11 (1H, d, J= 8.4Hz), 6.78 (1H, d, J=10.2 Hz), 6.71 (1H, s), 5.12 (1H, d, J=12.5Hz), 4.89 (1H, d, J=12.5 Hz), 4.67-4.55 (1H, m), 4.55 (1H, d J=12.5 Hz), 4.50 (1H, d, J=12.5 Hz), 4.48-4.29 (3H, m), 4.19-4.03 (2H, m), 4.02-4.92 (1H, m), 3.74-3.66 (1H, m), 3.07 (2H, d, J=7.4 Hz), 2.52 (1H, d, J=14.3 Hz), 2.32-2.06 (4H, m), 2.05-1.86 (1H, m), 1.71-1.52 (1H, m), 1.42 (9H, s), 1.06-0.89 (1H, m), 0.83-0.59 (6H, m); 13C NMR (CDCl3, 100 MHz) d = 175.5, 173.4, 171.5, 170.5, 170.4, 156.6, 137.9, 137.7, 137.1, 136.3, 129.2, 129.1, 128.5, 128.4, 127.9, 127.8, 127.7, 126.5, 82.1, 76.5, 71.6, 70.7, 66.5, 60.8, 57.8, 56.2, 53.1, 52.2, 37.0, 34.4, 33.3, 31.7, 27.9, 25.0, 24.6, 14.9, 10.9.

Step 2) The final product was obtained using the general Dess-Martin oxidation procedure as outlined above for compound **1052, figure 29** to produce **compound 1055**.

Synthesis of Compound 1056 as illustrated in figure 32. The following extended peptide isostere was synthesized on solid phase resin (MBHA) using standard peptide coupling conditions. The peptide was synthesized starting from the C-terminal end. *N*-BOC-Threonine was coupled to the resin (DCC, HOBT, DIEA), followed by deprotection of the amino group (TFA), and thorough washings with DMF and CH_2Cl_2. This standard coupling/ deprotection/ washing procedure was repeated seven times with the following *N*-BOC amino acids: (Gln, Ile, Pro, Phe (α-hydroxy acid,

Ala, Gln, Pro). Subsequent Dess-Martin oxidation was performed while the peptide was still bound to the solid support. The resin-bound peptide was suspended in CH_2Cl_2, and Dess-Martin periodinane was added (2.0 eq). The resin was washed with copious amounts of water and MeOH through a fritted funnel. This procedure was repeated to ensure complete oxidation to the α-keto-amide.

Cleavage of the peptide from the solid support was done in the standard anhydrous HF apparatus, and purified by reverse phase HPLC

Synthesis of 2(R),5(R)-Bis(hydroxymethyl)-3(R),4(S)-dihydroxy-N-(benzyloxycarbonyl)-pyrrolidine-2,3-benzylidene acetal compound 1057C Figure 33. To the substrate 2(R), 5(R)-Bis(hydroxymethyl)-3(R),4(S)-dihydroxy-N-(benzyloxycarbonyl)-pyrrolidine (267 mg, 0.90 mmol; synthesized according to the procedure in Slee et al. *J. Am. Chem. Soc.*, **1995**, *117*, 11867) dissolved in anhydrous DMF (1 mL) was added benzaldehyde dimethyl acetal (242 mL, 1.62 mmol, 1.8 eq.). A catalytic amount of pTSA was added and the solution was allowed to stir under argon for 6 hr, after which the solution was partitioned between H2O (30 mL)and EtOAc (30 mL), and the aqueous layer was extracted with EtOAc (2 x 30 mL). The pooled organic layers were washed with brine (20 mL), dried over MgSO4, filtered, and concentrated to yield a crude yellow oil. Subsequent flash column purification, eluting with 50% ethyl acetate in hexanes yielded a white foam **1057C** (246 mg, 71%).

Synthesis of compound 1057D as illustrated in Figure

33.

Step 1) To the 2(R), 5(R)-Bis(hydroxymethyl)-3(R),4(S)-dihydroxy-N-(benzyloxycarbonyl)-pyrrolidine-2,3-benzylidene acetal compound **1057C** (784 mg, 2.04 mmol) in anhydrous DMF (4 mL) was added TBDMSCl (316 mg, 2.09 mmol, 1.02 eq.), triethylamine (310 mL, 2.24 mmol, 1.1 eq.), and a catalytic amount of DMAP. The solution was stirred under argon for 18 hr, and then the solution was partitioned between EtOAc (40 mL) and H2O (30 mL). The aqueous layer was extracted with EtOAc (2 x 40 mL), and the organic layers were washed with sat'd NH4Cl (aq.) (30 mL), brine (30 mL), dried over MgSO4, filtered, and concentrated in vacuo to yield a crude yellow oil. (914 mg, 90%)

Step 2) The crude yellow oil was redissolved in 5 mL of anhydrous DMF (5 mL), and the solution was cooled to 0°C in an ice bath. NaH (52 mg, 2.16 mmol, 2.2 eq.), and benzyl bromide (0.128 mL, 1.08 mmol, 1.1 eq.) were added and the mixture was allowed to stir at 0°C for 1 hr. The reaction was quenched with sat'd NH4Cl (5 mL), and then EtOAc (40 mL) was added. The organic layer was washed with sat'd NaHCO3(aq.) (30 mL), water (2 x 30 mL), and brine (30 mL). After drying over MgSO4, filtration, and concentration in vacuo, the crude product was isolated as a yellow oil .

Synthesis of compound 1057E as illustrated in Figure 33.

Step 1) To the crude yellow oil (0.982 mmol) was added TBAF (1M in THF) (1.9 mL, 1.9 mmol, 2 eq.), and the solution was stirred 2 hr at ambient temperature.

The solution was concentrated in vacuo and immediately applied to a short silica gel column, eluting with 33% ethyl acetate in hexanes. The product was isolated as a white foam (443 mg, 95%). Step 2) The white foam (0.191 mmol) was dissolved in CH_2Cl_2 (1 mL) and Dess Martin periodinane (112 mg, 2.0 mmol, 2.0 eq.) was added. After 1 hr, sodium thiosulfate (500 mg) and H2O (30 mL) were added, and the aqueous layer was extracted with EtOAc (3 x 30 mL), dried over MgSO4, filtered, and concentrated to yield a crude product which was dissolved in tert-butanol (2.5 mL). To this solution, 2-methyl-2-butene (2M solution) (2 mL) was added and then a solution of sodium chlorite (199 mg, 1.75 mmol, 9.2 eq.) and NaH2PO4 (354 mg, 1.32 mmol, 6.9 eq.) in H2O (1 mL) was added dropwise to the stirring substrate solution. After 1 hr, TLC indicated that the reaction was complete and the volatiles were removed in vacuo, and then remaining aqueous layer was extracted with diethyl ether (3 x 20 mL).

Synthesis of Compound 1057 F as illustrated in Figure 33. As illustrated in figure33 the substrate **1057E** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. tertbutyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3

x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product

Synthesis of Compound 1057G as illustrated in Figure 33. Reductive cleavage of the benzylidene ring is done using 1.1 equivalents DIBAL in 1.0 THF solution at 0 °C for 1 hour. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desiredproduct

Synthesis of Compound 1057H as illustrated in Figure 33. Deprotection of the Cbz group will be achieved selectively (in the presence of benzyl groups) using 0.10 equivalents palladium hydroxide on carbon, as described in the procedure above for compound **1055C, Figure 31.**

Synthesis of Compound 1057 as illustrated in Figure 33.

As illustrated in figure 33, CB-Phe(α-OH) **11** (70 mg, 0.213 mmol), is dissolved in dry DMF (3 mL). HOBT, 1-hydroxybenzotriazole hydrate (31 mg, 0.22 mmol), EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol), DIEA, diisopropylethylamine, (122 µl, 0.703 mmol) are added and the mixture is stirred for 30 minutes at room temperature. The secondary amine **1057H** (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N HCl $_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product which is directly carried on to the next step for oxidation of the secondary alcohol as follows. The secondary alcohol (21 mg, 0.044 mmol) is dissolved in dry CH_2Cl_2 (2 mL), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture is stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 mL) and quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 mL) and sodium thiosulfate. The aqueous phase is extracted with ethyl acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in

hexane gives the respective product **1057** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%) as a colorless oil.

Synthesis of N-(benyloxycarbonyl)-3,4-didehydro-L-proline ethyl ester compound 1058C as illustrated in Figure 34. Synthesized according to the procedure from J. Org. Chem. 1994, 59, 5192.

Synthesis of N-(benzyloxycarbonyl)-(3R,4S)-dihydroxy-L-proline ethyl ester compound 1058D as illustrated in Figure 34.

To a solution of substrate (600 mg, 2.18 mmol) in *t*-butanol/ H_2O (10 mL: 10 mL) was added AD-mix-b (3.0g) and methanesulfonamide (290 mg, 3.05 mmol, 1.4 eq.), and reaction was stirred at 4°C for 48 hr. The aqueous phase is extracted with ethyl acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gives the respective product **1058D** as a colorless oil.

Synthesis of N-(benzyloxycarbonyl)-(3R,4S)-dihydroacetal-L-proline ethyl ester compound 1058E as illustrated in Figure 34. To the crude mixture of cis and trans - dihydroxy-L-proline ethyl ester was added acetone (10 mL), *p*TsA (a spatula-full), and $CuSO_4$ (spatula-full) and the mixture was refluxed and stirred for 24 h to give the corresponding acetonides. The aqueous phase is extracted with ethyl

acetate (3 x 20 mL). The combined organic extracts washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gives the respective product **1058E** as a colorless oil.

Synthesis of (3R,4S)-dihydroacetal-L-proline tert-butyl amide compound 1058F as illustrated in Figure 34

As illustrated in figure 34 the substrate **1058E** (70 mg, 0.213 mmol), is dissolved in dry methylene chloride (3 mL). EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, (43 mg, 0.224 mmol) is added and the mixture is stirred for 30 minutes at room temperature. *tert*-butyl amine (73 mg, 0.255 mmol) is added and the reaction stirred for 18 hours. The reaction mixture is diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase is extracted with ethyl acetate (3 x 10 mL). The combined organic phases are then washed with water (2 x 5 mL), 1 N $HCl_{(aq.)}$ (5 mL), saturated sodium bicarbonate solution $_{(aq.)}$ (50 mL), water (5 mL), brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography, eluting with 1:1 ethyl acetate/hexane gives the desired coupled product Step 2) The product was dissolved in MeOH (0.10 M) and a catalytic amount of 10% palladium on carbon was added. The mixture was stirred vigorously under a balloon of hydrogen until TLC indicated that the reaction was complete. Filtration and concentration afforded a clean product **1058F**.

Synthesis of compound 1058 as illustrated in Figure 34

To the pyrrolidine derivative **1058F** (20 mg, 0.091 mmol) was added dry methanol (2 mL), Cbz-phenylalanyl epoxide **21** (27 mg, 0.091 mmol, 1.0 eq.) and triethylamine (14 µL, 0.100mmol, 1.1eq.). The solution was refluxed for 32 h, and then concentrated *in vacuo*. Flash chromatography, eluting with ethyl acetate provides the desired product as a clear oil to give respectively hydroxylethyl amine derivative **1058**.

Preparation, Assay and Inhibition Analysis of the HIV Protease

Plasmid pLAC-PRO 6.5 which contains the *lac*Z-protease fusion construct under the control of the *rac* promoter (composed of the *E. coli* ribosomal RNA promoter fused to *lac* operator) was prepared and transformed to *E. coli* JM103. To induce *lac*Z-*pro* synthesis, overnight culture of JM103 harboring pLAC-PRO 6.5 (PRO 6-5) was diluted 50 fold with LB broth containing 300 g/mL ampicillin. Cells were grown at 37°C for 1 h to A_{600} = 0.2-0.3, lactose (0.4% final concentration) was then added to induce the synthesis of *lac*Z-protease fusion protein. After overnight growth, cells were harvested by centrifugation in a Sorvall GSA rotor at 6,000 rpm for 7 minutes. Cell paste from 250 mL culture was suspended in 10 mL lysis buffer (50 mM Tris, 2 mM EDTA, 2 mM DTT, 100 mM NaCl, pH 7.5) containing 200 g/mL lysozyme and stood at 4°C overnight. The cell suspension was

subsequently sonicated over a salted ice bath for 10 times with 10-15 pulses each at the maximal power output of the sonifier (Branson 450). Care was taken during sonication to keep the temperature of cell suspension below 10°C. The sonicates were then centrifuged at 6k rpm in a GSA rotor for 10 minutes. The majority of the *lacZ-pro* fusion proteins were found in the pellet. The insoluble *lacZ-pro* proteins were solubilized and unfolded in an aqueous solution containing 8M urea, and 50 mM dithiothreitol (DTT) or 1% 2-mercaptoethanol at concentrations of 0.2 mg/mL to 0.4 mg/mL. To refold the *lacZ-pro* proteins, the protein suspension were diluted with 12 volumes of 10 mM Tris at pH 6.0 and incubated at room temperature overnight (final protein concentration at 15 g/mL to 30 g/mL). Under these conditions, the *lacZ-pro* proteins underwent auto-proteolysis to yield the mature 10 kDa protease.

The renatured *lacZ*-protease fusion protein mixture was prepared as described above and freeze-dried in a lyophilizer overnight, resuspended in H_2O, and dialyzed against 10 mM Tris buffer at pH 8.0 overnight to remove urea. Under these conditions the mature HIV protease forms an insoluble precipitate and can be readily purified by low speed centrifugation at 2,000xg for 20 min. The precipitated protease was redissolved in 8M urea, 10 mM Tris, pH 8.0, 1 mM DTT and passed through a DEAE-Sephacel column or a Pharmacia monoQ column in the same buffer. The flow through fraction was collected and dialyzed against 10 mM Tris, pH 8.0, 1 mM DTT, and concentrated by lyophilization. Both SDS gel electrophoresis and immunoblot analysis and amino

acid analysis showed homogeneity of the protease. This procedure routinely gave 2-3 mg of the mature protease per gram of *E. coli* cells.

The synthetic HIV-protease containing a thioester linkage available from CalBiochem (San Diego) can also be used. The enzyme activity was assayed with the fluorogenic substrate Ac-Thr-Ile-Nle-Phe(P-NO_2)-Gln-Arg-NH_2 (from CalBiochem, San Diego) according to the procedure described in the literature. Inhibition analysis was performed in the presence of the inhibitor and expressed with IC_{50} (the concentration of inhibitor that causes 50% inhibition of the enzyme activity).

What is claimed is:

1. A method for identifying a drug candidate as an HIV protease inhibitor potentially resistive against loss of inhibitory activity due to development of resistant strains of HIV, the method comprising the following steps:

Step A: determining whether the drug candidate has a binding activity with respect to HIV protease of less than 1 µM;

Step B: determining whether the drug candidate has an inhibitory activity with respect to HIV protease of less than 1 µM;

Step C: determining whether the drug candidate has a binding activity with respect to FIV protease of less than 1 µM;

Step D: determining whether the drug candidate has an inhibitory activity with respect to FIV protease of less than 1 µM; and then

Step E: if, in said Steps A, B, C, and C, the drug candidate is determined to have binding and inhibitory activities with respect to both HIV protease and FIV protease of less than 1 µM, then selecting the drug candidate as the HIV protease inhibitor potentially resistive against loss of inhibitory activity due to development of resistant strains of HIV.

2. A method for synthesizing a drug candidate for inhibiting HIV protease, the drug candidate including an N-terminus, a C-terminus, and an α-keto amide core structure linking the N-terminus and the C-terminus, the N-terminus incuding an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrpsine, the aromatic amino acid including a carbonyl group for linking to and incorporation into the α-keto amide core structure, the C-terminus including a heterocyclic ring having a ring nitrogen and one or more substitutions, the ring nitrogen of the C-terminus for linking to and incorporation into the α-keto amide core structure, the method comprising the following steps:

Step A: providing an N-terminus precursor identical to the N-terminus except that the carbonyl group is replaced by an α-hydroxyl acid group;

Step B: providing a C-terminus precursor identical to the C-terminus except that the ring nitrogen forms a secondary amine;

Step C: coupling the N-terminus precursor of said Step A to the C-terminus precursor of said Step B to form a drug candidate precursor indentical to the drug candidate except that the α-keto amide core structure of the drug candidate is replaced by an α-hydroxyl amide core stucture linking and incorporating the carbonyl group of the N-terminus and the ring nitrogen of the C-terminus; and then

Step D: oxidizing the α-hydroxyl amide core stucture of the drug candidate precursor of said

Step C for forming the α-keto amide core structure and the drug candidate.

3. A method for synthesizing a library of nxm drug candidates for inhibiting HIV protease, each of the nxm drug candidates including an N-terminus selected from **n** N-termini where **n** is two or greater, a C-terminus selected from **m** C-termini where m is two or greater, and an α-keto amide core structure linking the N-terminus and the C-terminus, each of the **n** N-termini incuding an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrosine, the aromatic amino acid including a carbonyl group for linking to and incorporating into the α-keto amide core structure, each of the **m** C-termini including a heterocyclic ring having a ring nitrogen and one or more substitutions, the ring nitrogen of the C-terminus for linking to and incorporating into the α-keto amide core structure, the method comprising the following steps:

Step A: providing **n** N-terminus precursors identical in structure to the **n** N-termini except that the carbonyl group of the N-termini is replaced by an α-hydroxyl acid group within the N-terminus precursors;

Step B: providing **m** C-terminus precursors identical in structure to the **m** C-termini except that the ring nitrogen of the C-termini forms a secondary amine within the C-terminus precursors;

Step C: providing nxm reaction vessels;

Step D: loading each of the **n** N-terminus precursors into **m** of the reaction vessels of said Step C;

Step E: loading each of the **m** C-terminus

precursors into **n** of reaction vessels of said Step D for forming nxm admixtures of N-terminus precursor and C-terminus precursors; then

Step F: within each of the nxm admixtures of said Step E, coupling the N-terminus precursor to the C-terminus precursor to form nxm drug candidate precursors identical to the nxm drug candidates except that the α-keto amide core structure of the nxm drug candidates is replaced by an α-hydroxyl amide core stucture linking and incorporating the carbonyl group of the N-terminus and the ring nitrogen of the C-terminus; and then

Step G: within each of the nxm reaction vessels, oxidizing the α-hydroxyl amide core stucture of each of the nxm drug candidate precursors of said Step F for forming the α-keto amide core structure and the library of nxm drug candidates.

4. A method for synthesizing a library of nxm drug candidates for inhibiting HIV protease, each of the nxm drug candidates including an N-terminus selected from **n** N-termini where **n** is two or greater, a C-terminus selected from **m** C-termini where **m** is two or greater, and a hydroxyethylamine core structure linking the N-terminus and the C-terminus, each of the **n** N-termini incuding an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrosine, the aromatic amino acid including a hydroxyethyl group *in lieu* of a carbonyl group for linking to and incorporating into the hydroxyethylamine core

structure, each of the **m** C-termini including a heterocyclic ring having a ring nitrogen and one or more substitutions, the ring nitrogen of the C-terminus for linking to and incorporating into the hydroxyethylamine core structure, the method comprising the following steps:

Step A: providing **n** N-terminus precursors identical in structure to the **n** N-termini except that the hydroxyethyl group of the N-termini is replaced by an epoxide group within the N-terminus precursors;

Step B: providing **m** C-terminus precursors identical in structure to the **m** C-termini except that the ring nitrogen of the C-termini forms a secondary amine within the C-terminus precursors;

Step C: providing nxm reaction vessels;

Step D: loading each of the **n** N-terminus precursors into **m** of the reaction vessels of said Step C;

Step E: loading each of the **m** C-terminus precursors into **n** of reaction vessels of said Step D for forming nxm admixtures of N-terminus precursor and C-terminus precursors; then

Step F: within each of the nxm admixtures of said Step E, coupling the N-terminus precursor to the C-terminus precursor for forming the library of nxm drug candidates.

5. A library of nxm drug candidates for inhibiting HIV protease, each of the nxm drug candidates including an N-terminus selected from **n** N-termini

where **n** is two or greater, a C-terminus selected from **m** C-termini where **m** is two or greater, and an α-keto amide core structure linking the N-terminus and the C-terminus, each of the **n** N-termini incuding an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrosine, the aromatic amino acid including a carbonyl group for linking to and incorporating into the α-keto amide core structure, each of the **m** C-termini including a heterocyclic ring having a ring nitrogen and one or more substitutions, the ring nitrogen of the C-terminus for linking to and incorporating into the α-keto amide core structure.

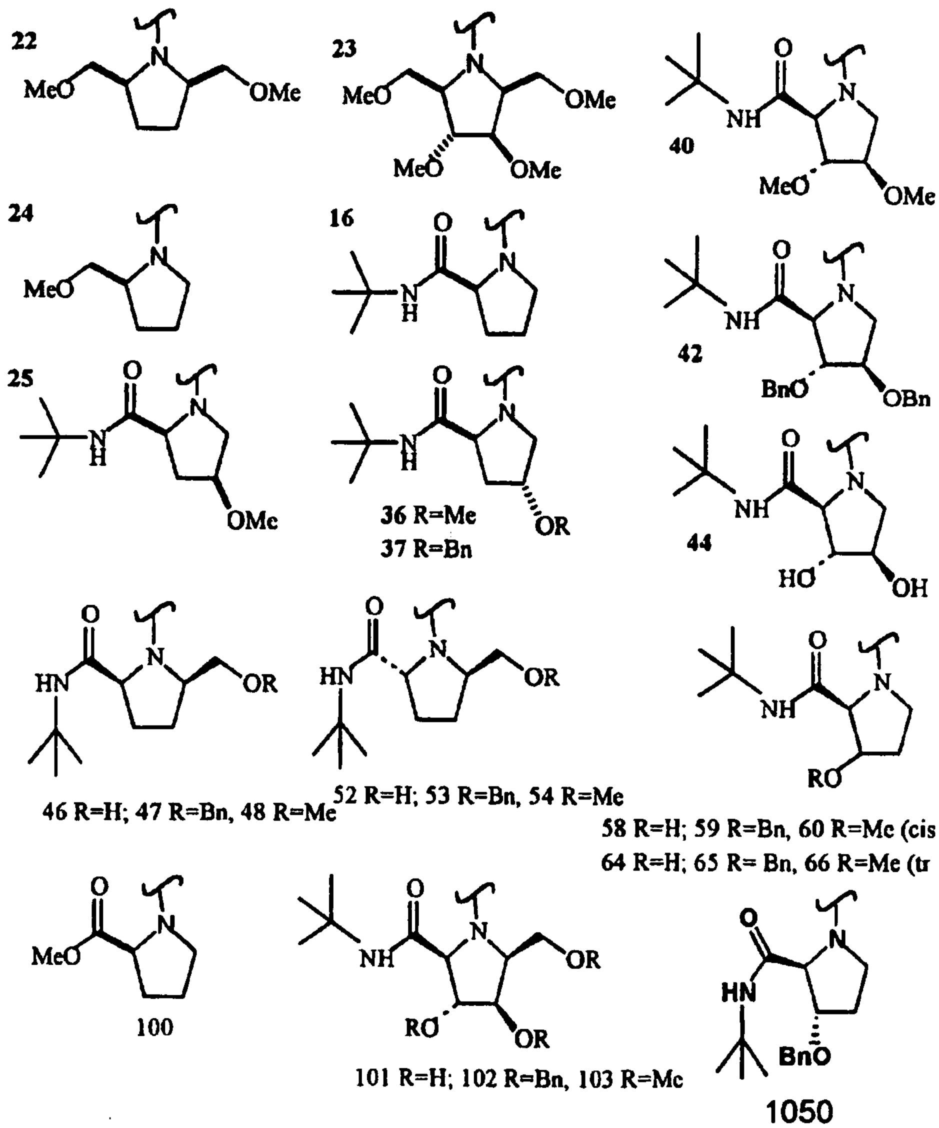
6. A library of nxm drug candidates for inhibiting HIV protease, each of the nxm drug candidates including an N-terminus selected from **n** N-termini where **n** is two or greater, a C-terminus selected from **m** C-termini where **m** is two or greater, and a hydroxyethylamine core structure linking the N-terminus and the C-terminus, each of the **n** N-termini incuding an aromatic amino acid residue selected from the group consisting of phenylalanine, tyrosine, and O-substituted tyrosine, the aromatic amino acid including a hydroxyethyl group *in lieu* of a carbonyl group for linking to and incorporating into the hydroxyethylamine core structure, each of the **m** C-termini including a heterocyclic ring having a ring nitrogen and one or more substitutions, the ring nitrogen of the C-terminus for linking to and incorporating into the hydroxyethylamine core structure.

7. An improved mechanism based inhibitor of HIV or FIV aspartyl protease of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus, the N-terminus incuding an aromatic amino acid residue linked to said core structure, the C-terminus including a heterocyclic ring including a ring nitrogen linked to said core structure, the core structure being isosteric with a scissile amide bond of a HIV or FIV aspartyl protease substrate, wherein the improvement comprises:

said core structure being an α-keto amide, and

the heterocyclic ring of said N-terminus being a pyrrolidine having at least one substituant other than carboxylic acid and carboxymethyl ester.

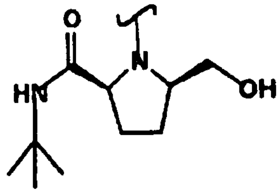
8. An improved mechanism based inhibitor of HIV or FIV aspartyl protease as described in claim 7 wherein said pyrrolidine is selected from the group represented by the following structures:



22; 23; 40; 24; 16; 42; 25; 36 R=Me, 37 R=Bn; 44

46 R=H; 47 R=Bn, 48 R=Me; 52 R=H; 53 R=Bn, 54 R=Me; 58 R=H; 59 R=Bn, 60 R=Me (cis; 64 R=H; 65 R= Bn, 66 R=Me (tr

100; 101 R=H; 102 R=Bn, 103 R=Me; 1050



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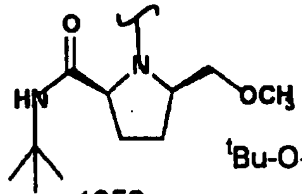

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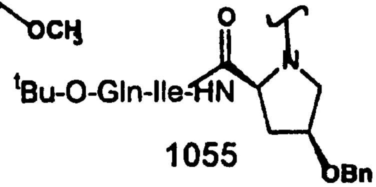

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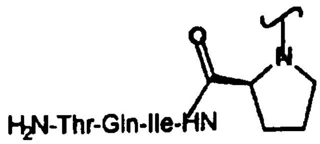

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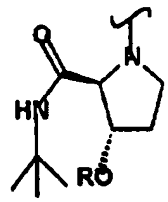
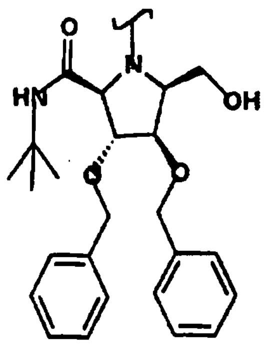
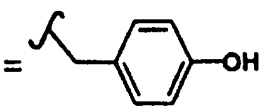
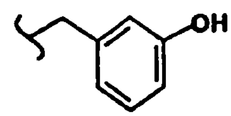
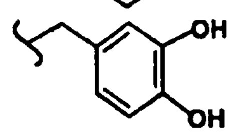




where:

1054 R = 

1059 R = 

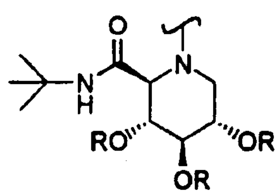
1060 R = 

9. An improved mechanism based inhibitor of HIV or FIV aspartyl protease of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus, the N-terminus incuding an aromatic amino acid residue linked to said core structure, the C-terminus including a heterocyclic ring including a ring nitrogen linked to said core structure, the core structure being isosteric with a scissile amide bond of a HIV or FIV aspartyl protease substrate, wherein the improvement comprises:

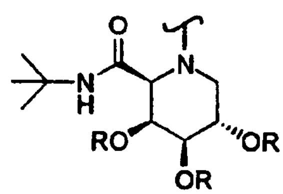
said core structure being an α-keto amide, and

the heterocyclic ring of said N-terminus being a piperadine or an azasugar.

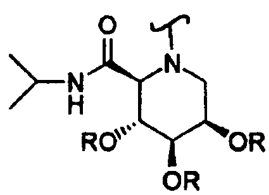
10. An improved mechanism based inhibitor of HIV or FIV aspartyl protease as described in claim 9 wherein said piperadine or azasugar is selected from the group represented by the following structures:



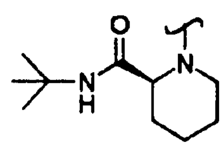
R = 85 H, 86 Me, 87 Bn



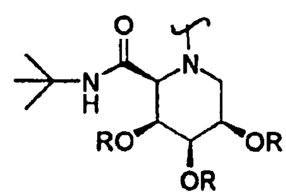
R = 88 H, 89 Me, 90 Bn



R = 91 H, 92 Me, 93 Bn



94



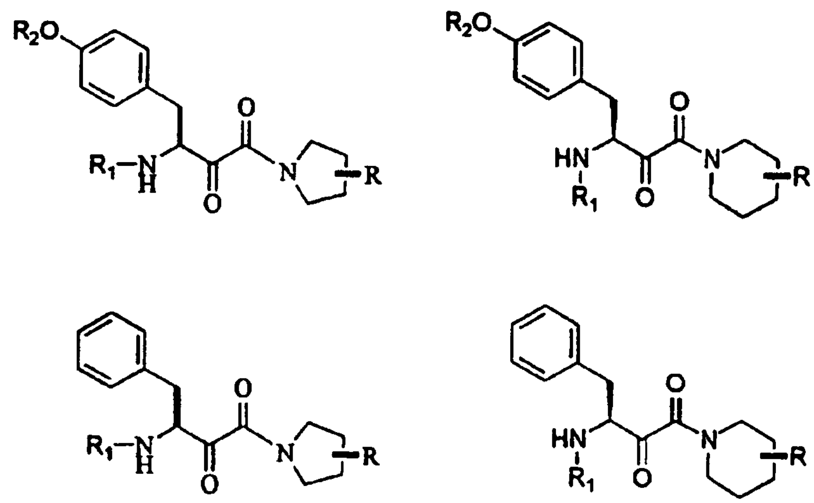
R = 95 H, 96 Me, 97 Bn

11. An improved mechanism based inhibitor of HIV or FIV aspartyl protease of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus, the N-terminus incuding an aromatic amino acid residue linked to said core structure, the C-terminus including a heterocyclic ring including a ring nitrogen linked to said core structure, the core structure being isosteric with a scissile amide bond of a HIV or FIV aspartyl protease substrate, wherein the improvement comprises:

said core structure being an α-keto amide, and

the aromatic amino acid of said C-terminus being selected from a group consisting of tyrosine having a protected amino, tyrosine having a protected amino and a substituted hydroxyl, and phenylalanine having a protected amino protected by carbobenzyloxy.

12. An improved mechanism based inhibitor of HIV or FIV aspartyl protease as described in claim 11 selected from the group represented by the following structures:



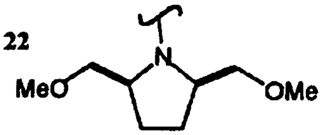
wherein R is selected from the group consisting of hydrogen, hydroxy, benzyloxy, alkyl$_{(C1-C4)}$-oxy, *o*-methoxy-benzyloxy, *m*-methoxy-benzyloxy, *p*-methoxy-benzyloxy, *o*-methoxy-nitrobenzyloxy, *m*-methoxy-nitrobenzyloxy, *p*-methoxy-nitrobenzyloxy, acetonide, benzylidene, 3-oxymethyl-catechol, 4-oxymethyl-catechol; R_1 is selected from the group consisting of carbobenzyloxy (CBZ), *tert*-butoxycarbonyl (*t*-BOC), acyl; R_2 is selected from the group consisting of hydrogen, benzyl, alkyl$_{(C1-C4)}$, *o*-methoxy-benzyl, *m*-methoxy-benzyl, *p*-methoxy-benzyl, *o*-methoxy-nitrobenzyl, *m*-methoxy-nitrobenzyl, *p*-methoxy-nitrobenzyl, 3-methylene-catechol, 4-methylene-catechol.

13. An improved mechanism based inhibitor of HIV or FIV aspartyl protease of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus, the N-terminus incuding an aromatic amino acid residue linked to said core structure, the C-terminus including a heterocyclic ring including a ring nitrogen linked to said core structure, the core structure being isosteric with a scissile amide bond of a HIV or FIV aspartyl protease substrate, wherein the improvement comprises:

said core structure being hydroxyethylamine, and

the heterocyclic ring of said N-terminus being a pyrrolidine having at least one substituant other than carboxylic acid and carboxymethyl ester.

14. An improved mechanism based inhibitor of HIV or FIV aspartyl protease as described in claim 13 wherein said pyrrolidine is selected from the group represented by the following structures:

22


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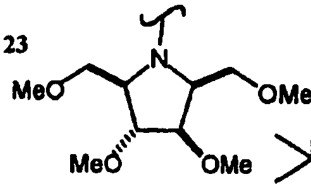

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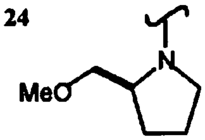

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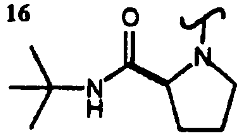
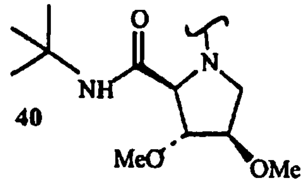


40


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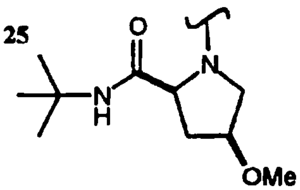
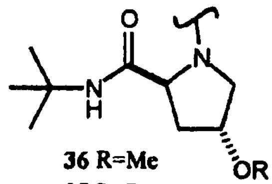
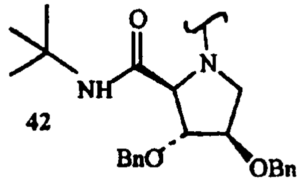
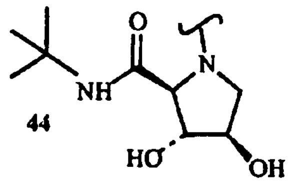
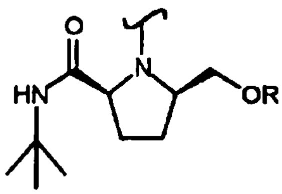
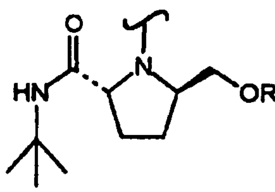


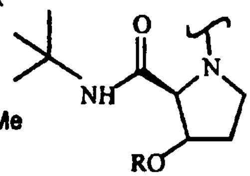

36 R=Me
37 R=Bn

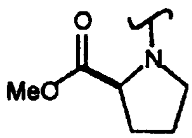
42


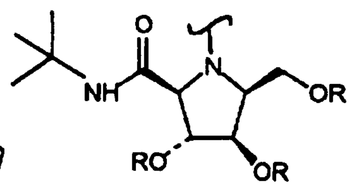
44


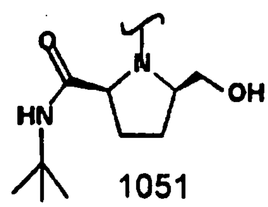

46 R=H; 47 R=Bn, 48 R=Me


52 R=H; 53 R=Bn, 54 R=Me

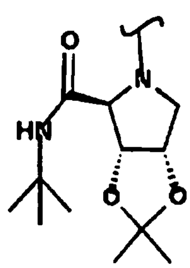

58 R=H; 59 R=Bn, 60 R=Me (cis)
64 R=H; 65 R= Bn, 66 R=Me (tra


100


101 R=H; 102 R=Bn, 103 R=Me


1051

and

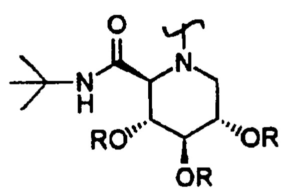

1058

15. An improved mechanism based inhibitor of HIV or FIV aspartyl protease of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus, the N-terminus incuding an aromatic amino acid residue linked to said core structure, the C-terminus including a heterocyclic ring including a ring nitrogen linked to said core structure, the core structure being isosteric with a scissile amide bond of a HIV or FIV aspartyl protease substrate, wherein the improvement comprises:

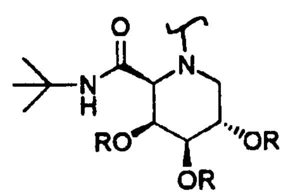
said core structure being hydroxyethylamine, and

the heterocyclic ring of said N-terminus being a piperadine or an azasugar.

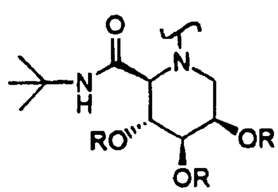
16. An improved mechanism based inhibitor of HIV or FIV aspartyl protease as described in claim 15 wherein said piperadine or azasugar is selected from the group represented by the following structures:



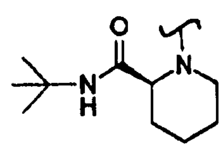
R = 85 H, 86 Me, 87 Bn



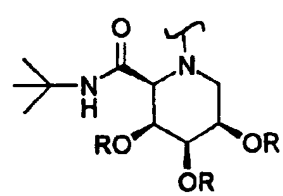
R = 88 H, 89 Me, 90 Bn



R = 91 H, 92 Me, 93 Bn



94

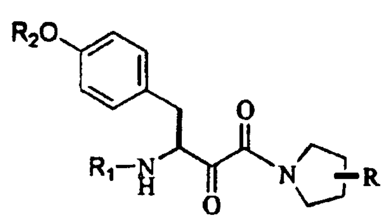


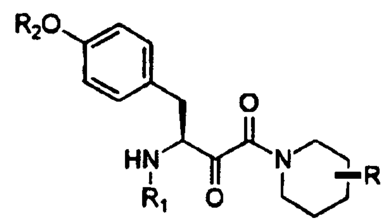
R = 95 H, 96 Me, 97 Bn

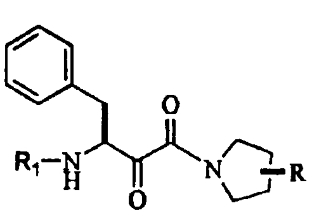
17. An improved mechanism based inhibitor of HIV or FIV aspartyl protease of a type having an N-terminus, a C-terminus, and a core structure for linking the N-terminus to the C-terminus, the N-terminus incuding an aromatic amino acid residue linked to said core structure, the C-terminus including a heterocyclic ring including a ring nitrogen linked to said core structure, the core structure being isosteric with a scissile amide bond of a HIV or FIV aspartyl protease substrate, wherein the improvement comprises:

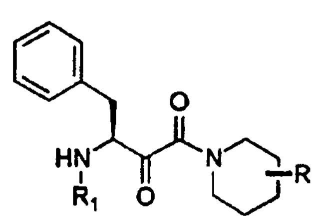
said core structure being hydroxyethylamine, and the aromatic amino acid of said C-terminus being selected from a group consisting of tyrosine having a protected amino, tyrosine having a protected amino and a substituted hydroxyl, and phenylalanine having a protected amino protected by carbobenzyloxy.

18. An improved mechanism based inhibitor of HIV or FIV aspartyl protease as described in claim 17 selected from the group represented by the following structures:









wherein R is selected from the group consisting of hydrogen, hydroxy, benzyloxy, alkyl$_{(C1-C4)}$-oxy, *o*-methoxy-benzyloxy, *m*-methoxy-benzyloxy, *p*-methoxy-benzyloxy, *o*-methoxy-nitrobenzyloxy, *m*-methoxy-nitrobenzyloxy, *p*-methoxy-nitrobenzyloxy, acetonide, benzylidene, 3-oxymethyl-catechol, 4-oxymethyl-catechol; R_1 is selected from the group consisting of carbobenzyloxy (CBZ), *tert*-butoxycarbonyl (*t*-BOC), acyl; R_2 is selected from the group consisting of hydrogen, benzyl, alkyl$_{(C1-C4)}$, *o*-methoxy-benzyl, *m*-methoxy-benzyl, *p*-methoxy-benzyl, *o*-methoxy-nitrobenzyl, *m*-methoxy-nitrobenzyl, *p*-methoxy-nitrobenzyl, 3-methylene-catechol, 4-methylene-catechol.

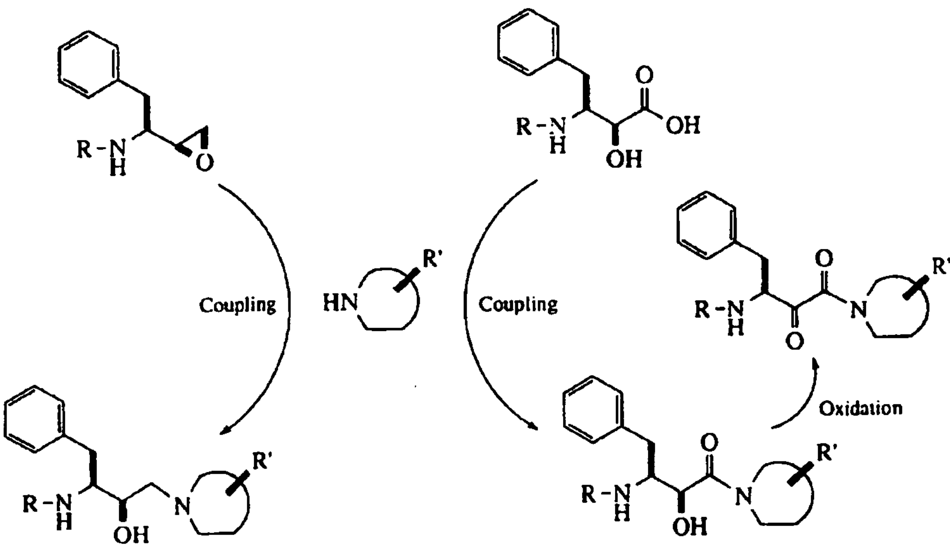
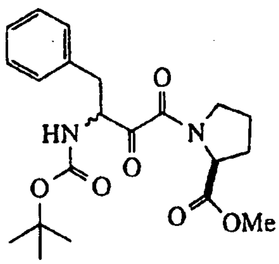
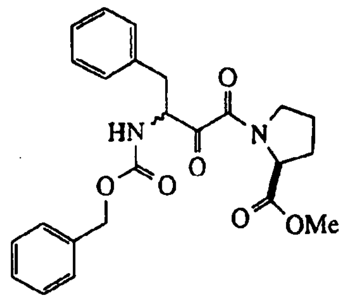

R-N
H
O
Coupling
HN
R'
Coupling
R-N
H
OH
O
OH
O
O
R-N
H
N
R'
Oxidation
R-N
H
OH
N
R'
O
R-N
H
OH
N
R'

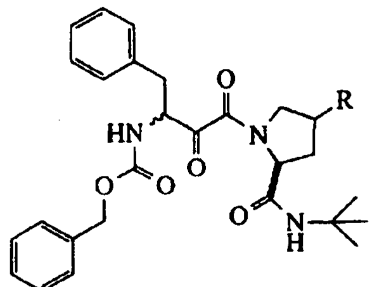
Figure 1



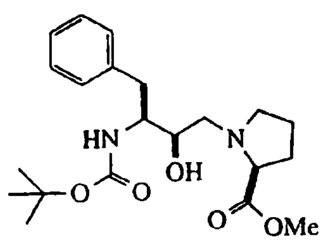
1a K_i = 6μM



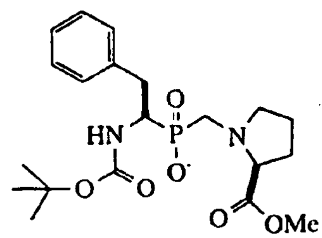
1b K_i = 405 nM



2 R = H; K_i = 214 nM
73 R = OBn (*cis*); K_i = 65 nM
74 R = OMe (*trans*); K_i = 220 nM
75 R = OBn (*trans*); K_i = 318 nM



K_i = >50 μM



K_i = 230 μM

Figure 2

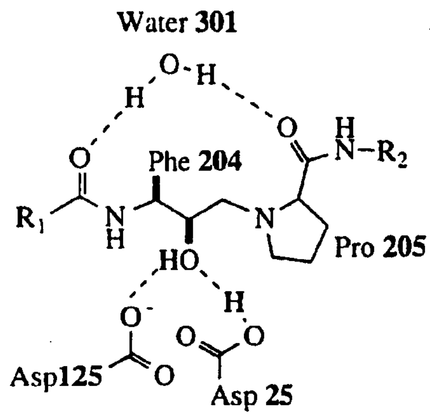

Water 301
O-H
H
O
H
N-R2
O
Phe 204
R1
N
H
N
Pro 205
HO
H
O-
O
O
Asp125
O
Asp 25

Figure 3

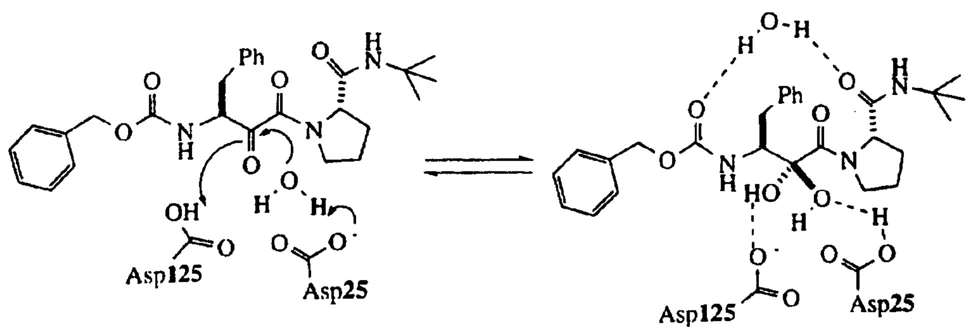
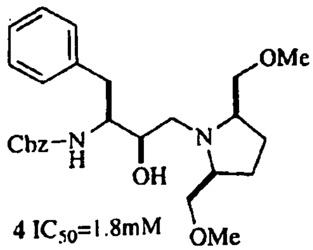
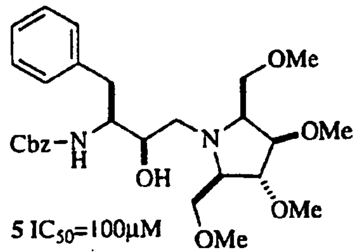

Ph
O
N
H
O
O
N
O
H
OH
H
O
H
H
Asp125
O
O
O
Asp25
⇌
H
O-H
H
O
Ph
O
N
H
HO
O
H
O
H
O
Asp125
O
O
Asp25

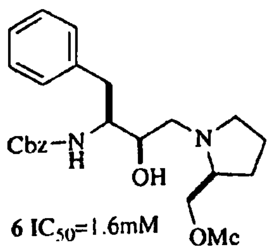
Figure 4



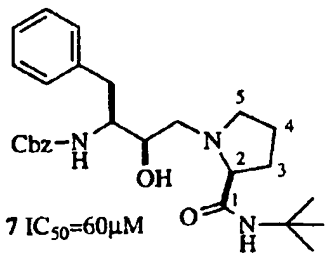
4 IC_{50}=1.8mM



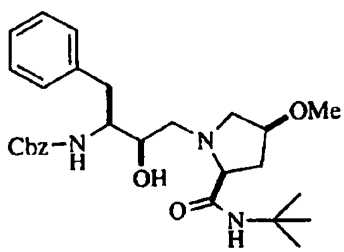
5 IC_{50}=100μM



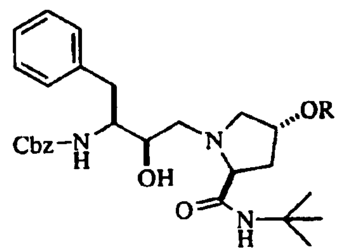
6 IC_{50}=1.6mM



7 IC_{50}=60μM



8 IC_{50}=17μM



38 (R=Me) IC_{50}=2.9μM
39 (R=Bn) IC_{50}=3.9μM

Figure 5

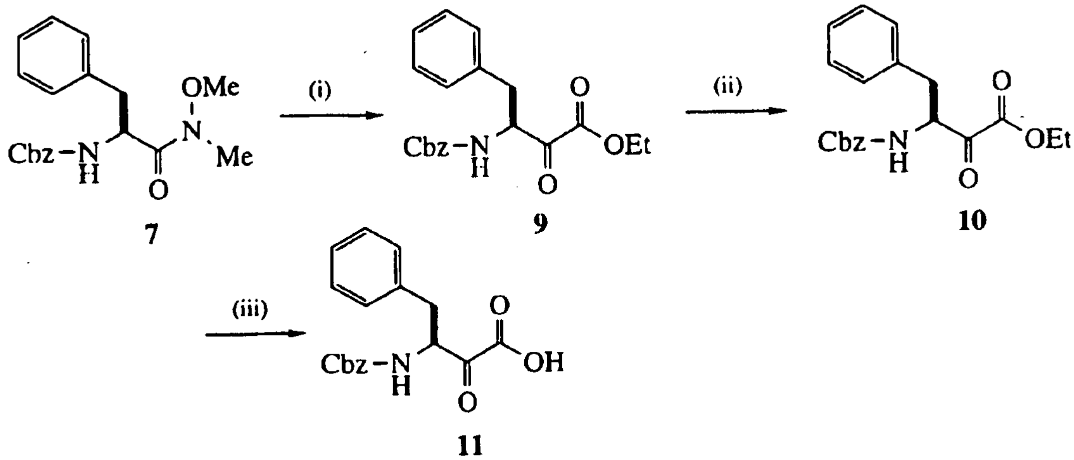


FIGURE 6

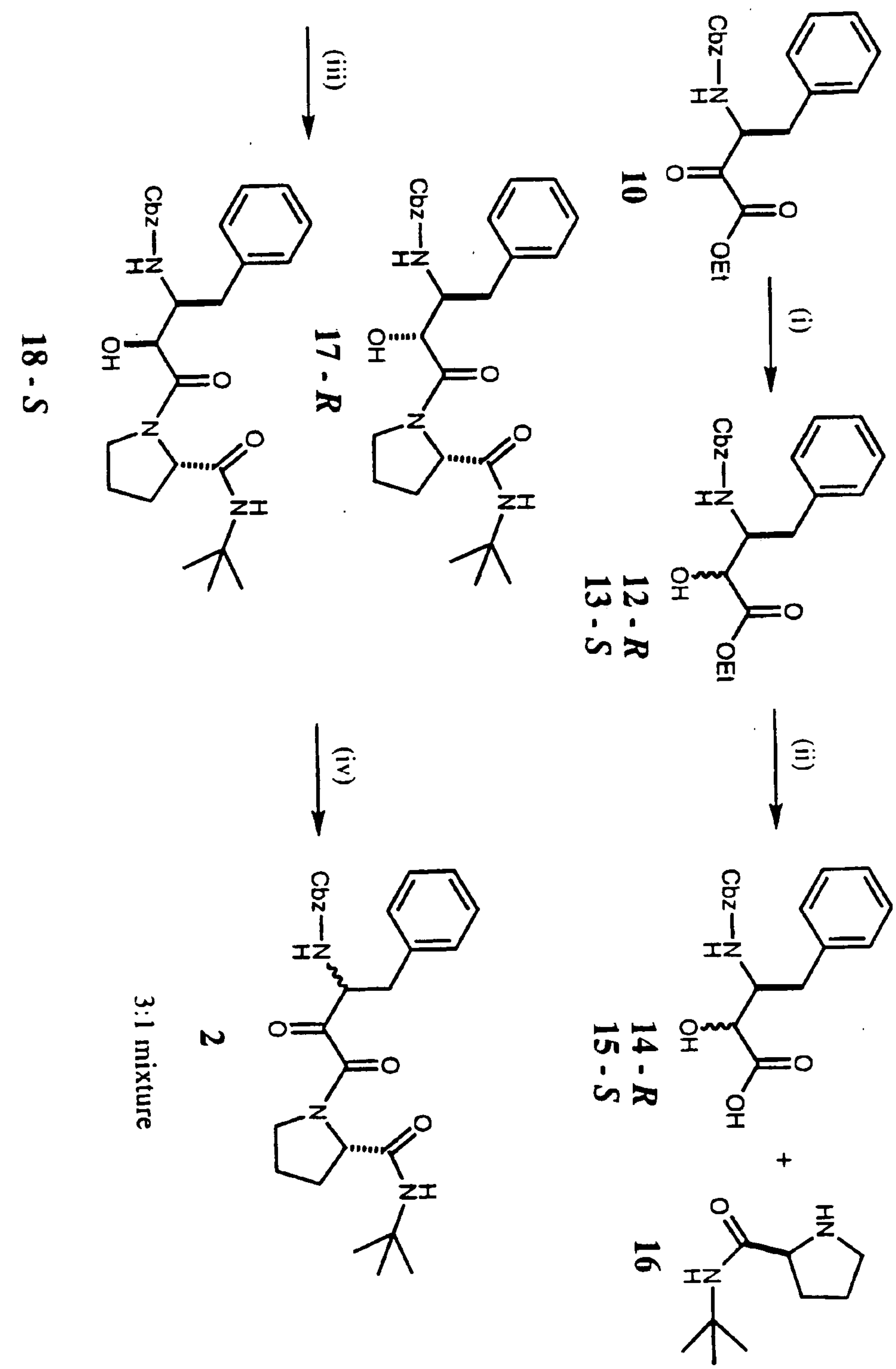


FIGURE 7

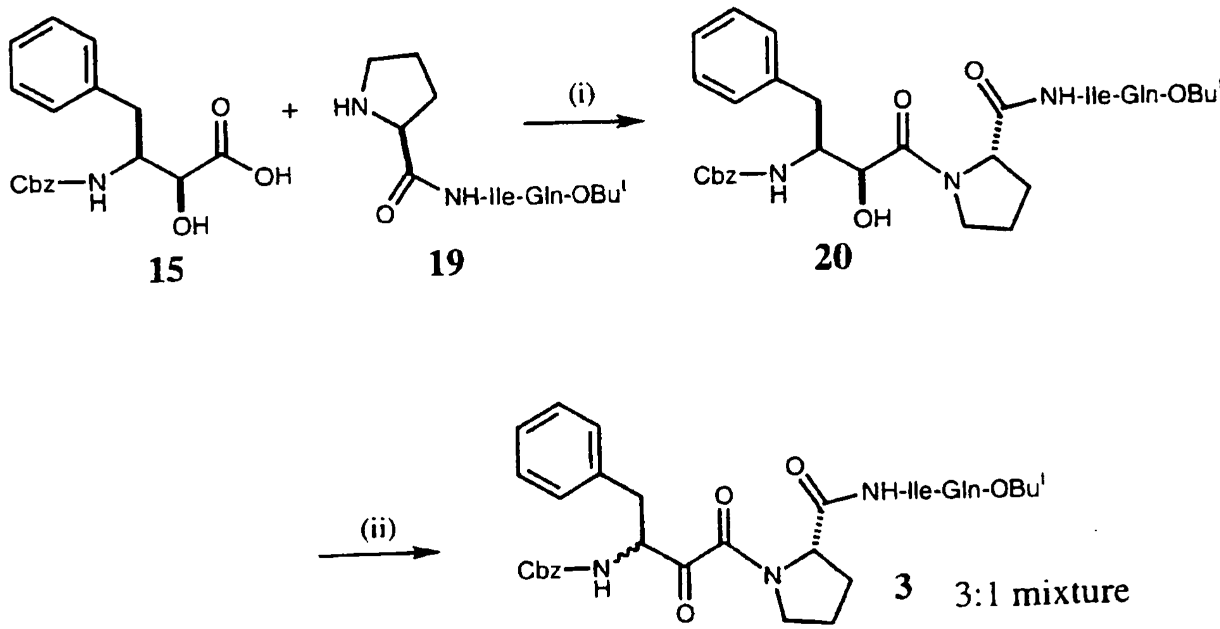
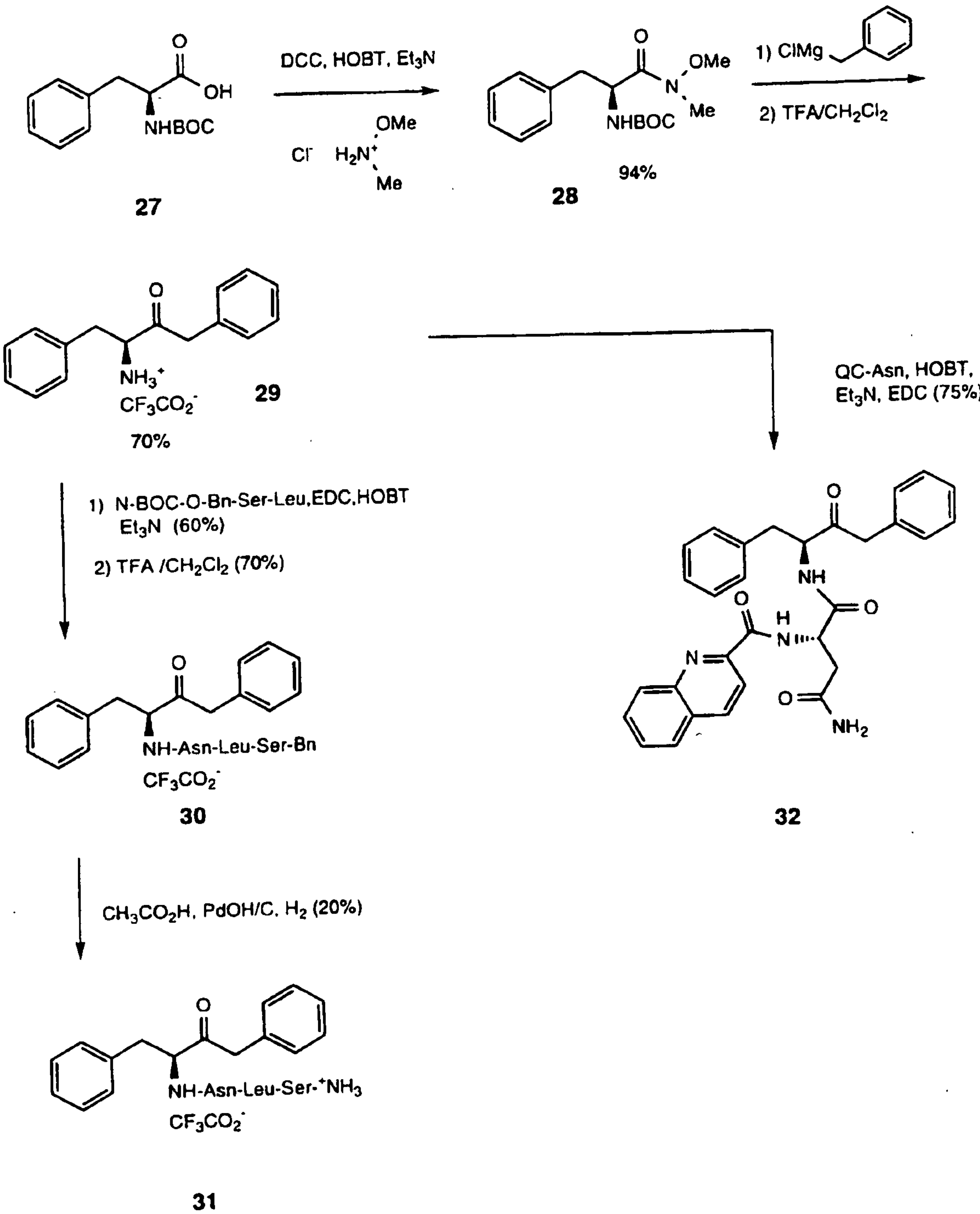
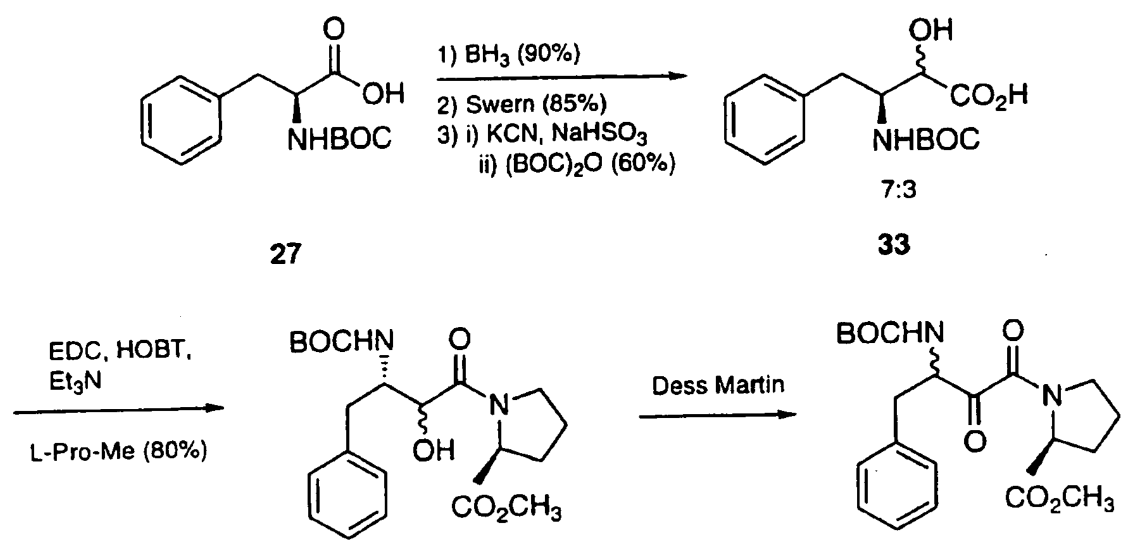


FIGURE 8



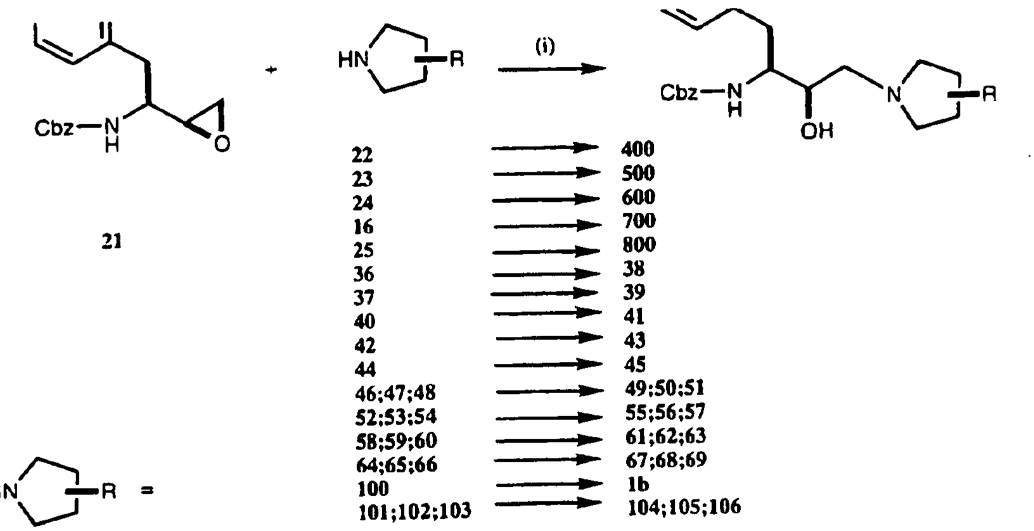
NHBOC
OH
27
DCC, HOBT, Et$_3$N
Cl$^-$ H$_2$N$^+$ OMe Me
OMe
N
Me
NHBOC
94%
28
1) ClMg
2) TFA/CH$_2$Cl$_2$
NH$_3^+$
CF$_3$CO$_2^-$
29
70%
QC-Asn, HOBT, Et$_3$N, EDC (75%)
1) N-BOC-O-Bn-Ser-Leu,EDC,HOBT Et$_3$N (60%)
2) TFA /CH$_2$Cl$_2$ (70%)
NH-Asn-Leu-Ser-Bn
CF$_3$CO$_2^-$
30
NH
H
N
NH$_2$
32
CH$_3$CO$_2$H, PdOH/C, H$_2$ (20%)
NH-Asn-Leu-Ser-$^+$NH$_3$
CF$_3$CO$_2^-$
31

FIGURE 9



27 33 34 35

FIGURE 10


Cbz—N H
O
21
HN R
(i)
Cbz—N H
OH
N R
22 → 400
23 → 500
24 → 600
16 → 700
25 → 800
36 → 38
37 → 39
40 → 41
42 → 43
44 → 45
46;47;48 → 49;50;51
52;53;54 → 55;56;57
58;59;60 → 61;62;63
64;65;66 → 67;68;69
100 → 1b
101;102;103 → 104;105;106
HN R =

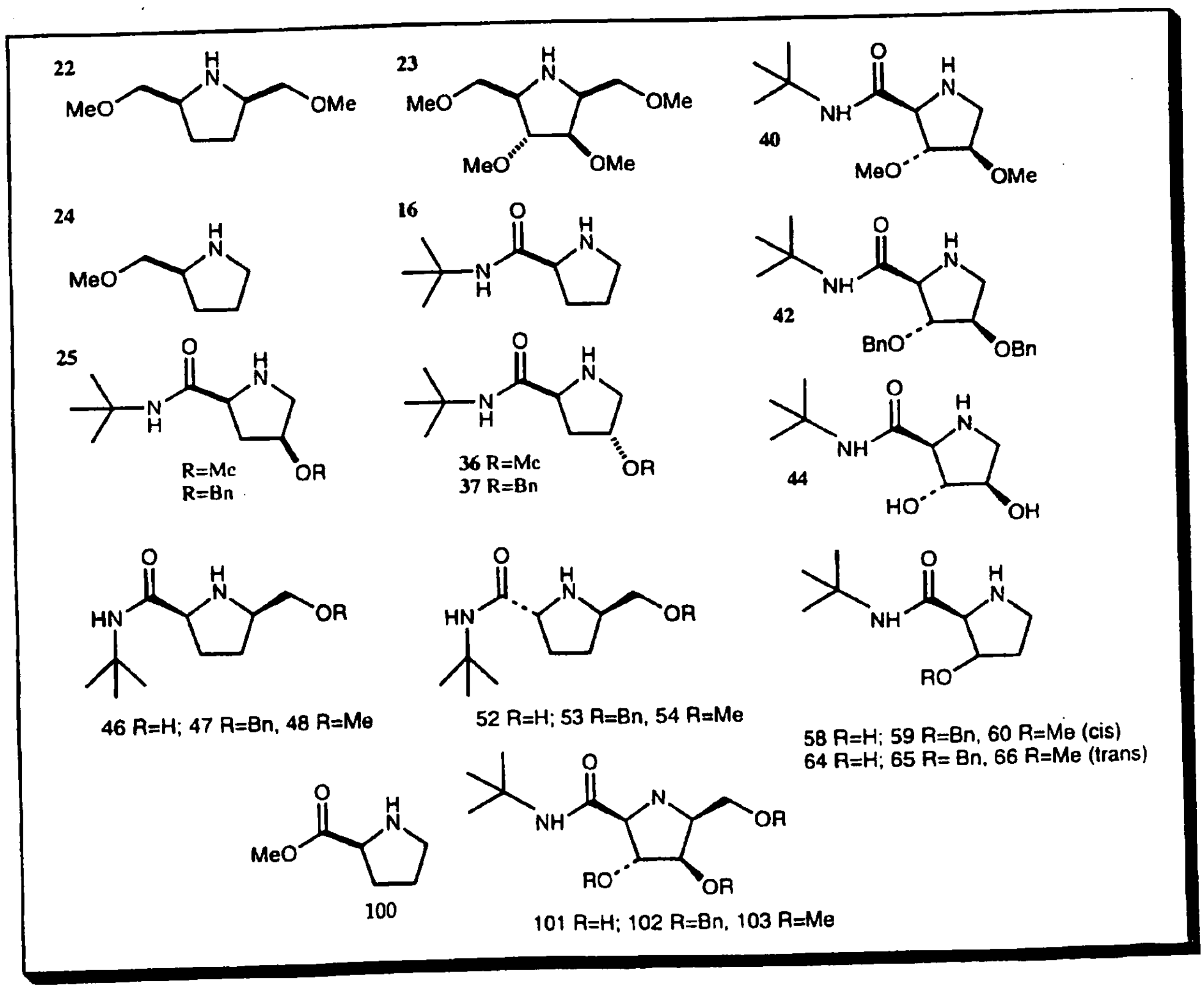
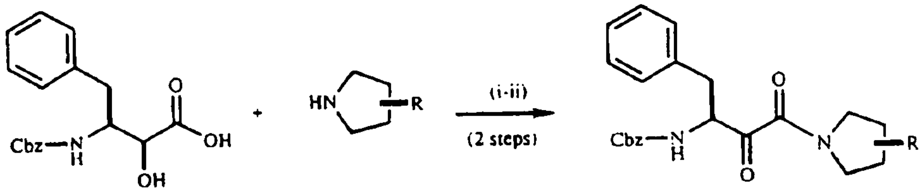
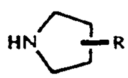

22
MeO OMe
23
MeO OMe
MeO OMe
40
NH
MeO OMe
24
MeO
16
N H
42
NH
BnO OBn
25
N H
R=Mc
R=Bn
OR
36 R=Mc
37 R=Bn
OR
44
NH
HO OH
HN
OR
46 R=H; 47 R=Bn, 48 R=Me
HN
OR
52 R=H; 53 R=Bn, 54 R=Me
NH
RO
58 R=H; 59 R=Bn, 60 R=Me (cis)
64 R=H; 65 R= Bn, 66 R=Me (trans)
MeO
100
NH
OR
RO OR
101 R=H; 102 R=Bn, 103 R=Me

FIGURE 11



15

22 → 70
23 → 900
24 → 71
16 → 2
25 → 73
36 → 74
37 → 75
40 → 76
42 → 77
44 → 78
46;47;48 → 79a;80a:81a
52;53;54 → 79b:80b;81b
58;59;60 → 82a;83a;84a
64;65;66 → 82b;83b;84b
100 → 1c
101;102:103 → 107;108;109

 =

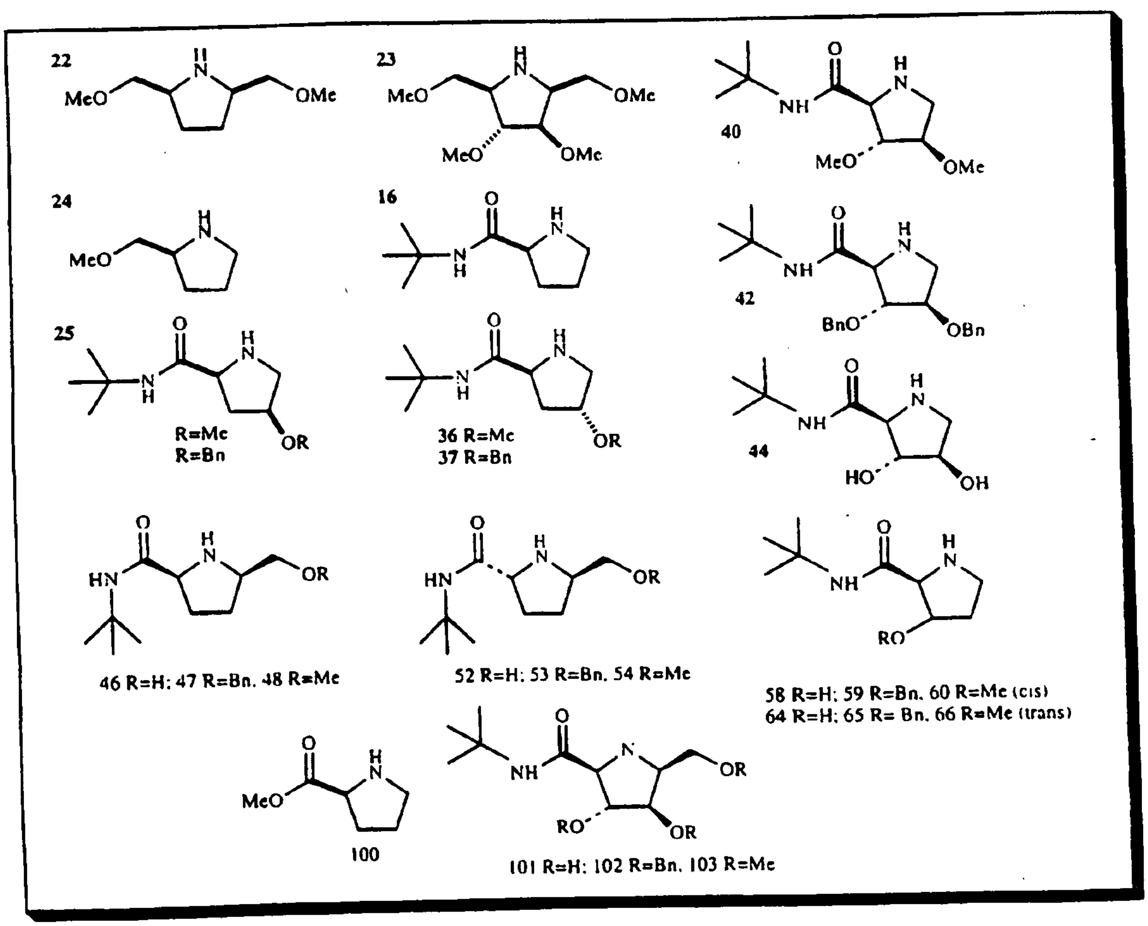


FIGURE 12

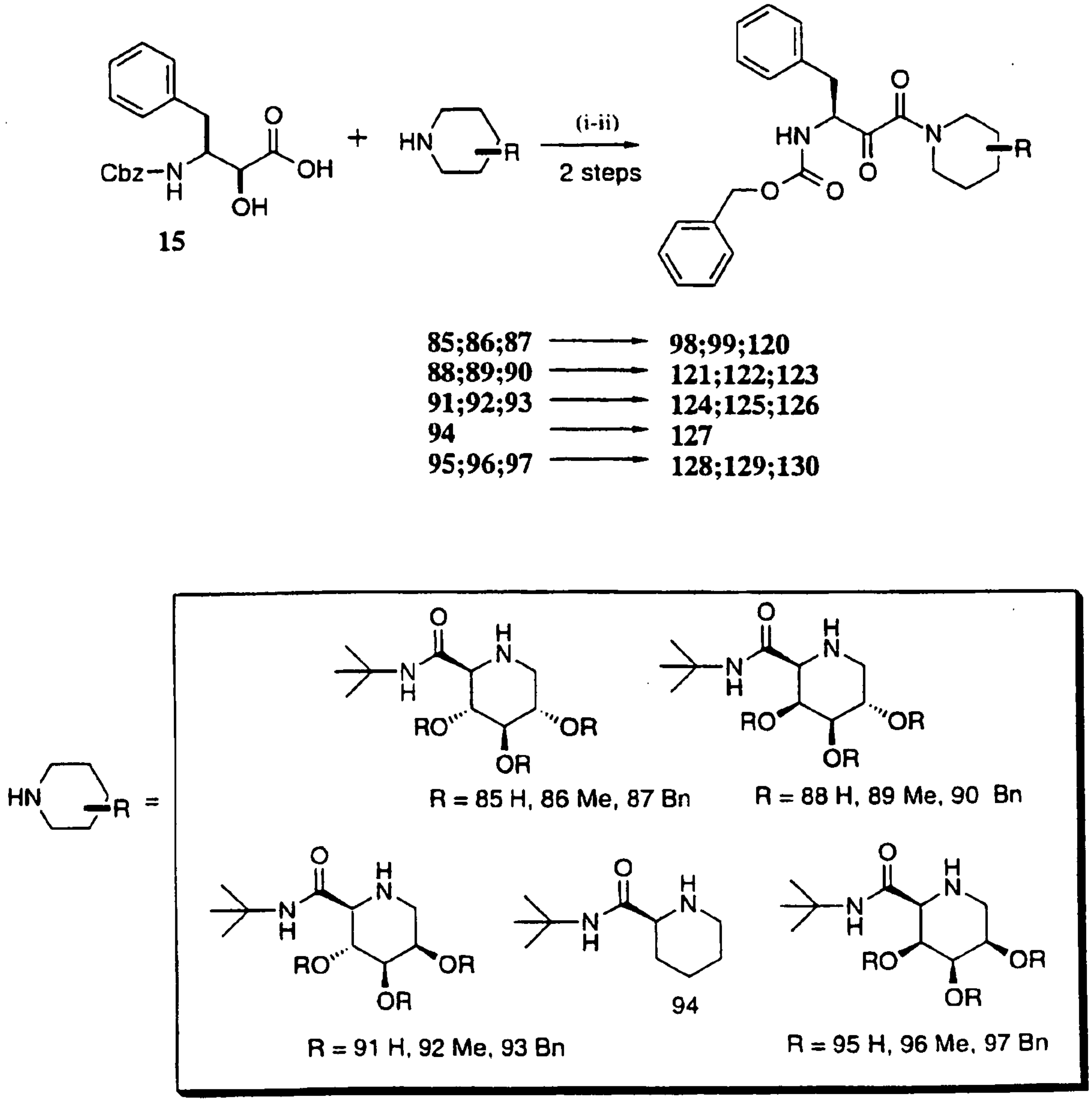
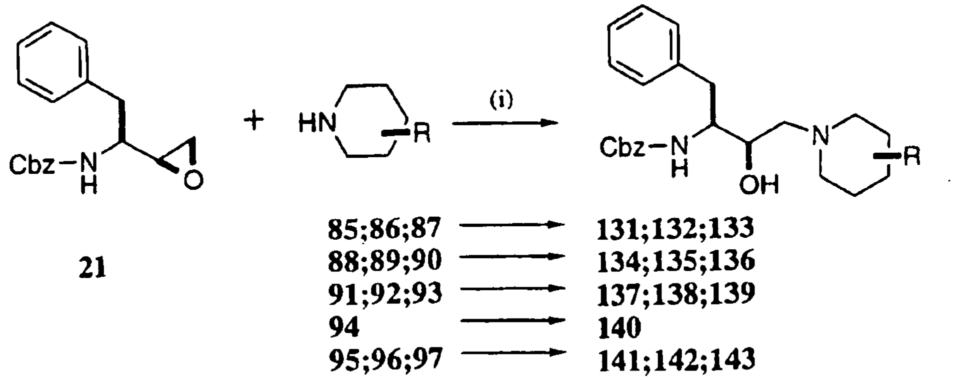

Cbz
OH
(i-ii)
2 steps
15
85;86;87 → 98;99;120
88;89;90 → 121;122;123
91;92;93 → 124;125;126
94 → 127
95;96;97 → 128;129;130
R = 85 H, 86 Me, 87 Bn
R = 88 H, 89 Me, 90 Bn
94
R = 91 H, 92 Me, 93 Bn
R = 95 H, 96 Me, 97 Bn

Figure 13


Cbz
N
H
O
21
HN
R
(i)
Cbz
N
H
OH
N
R
85;86;87 → 131;132;133
88;89;90 → 134;135;136
91;92;93 → 137;138;139
94 → 140
95;96;97 → 141;142;143

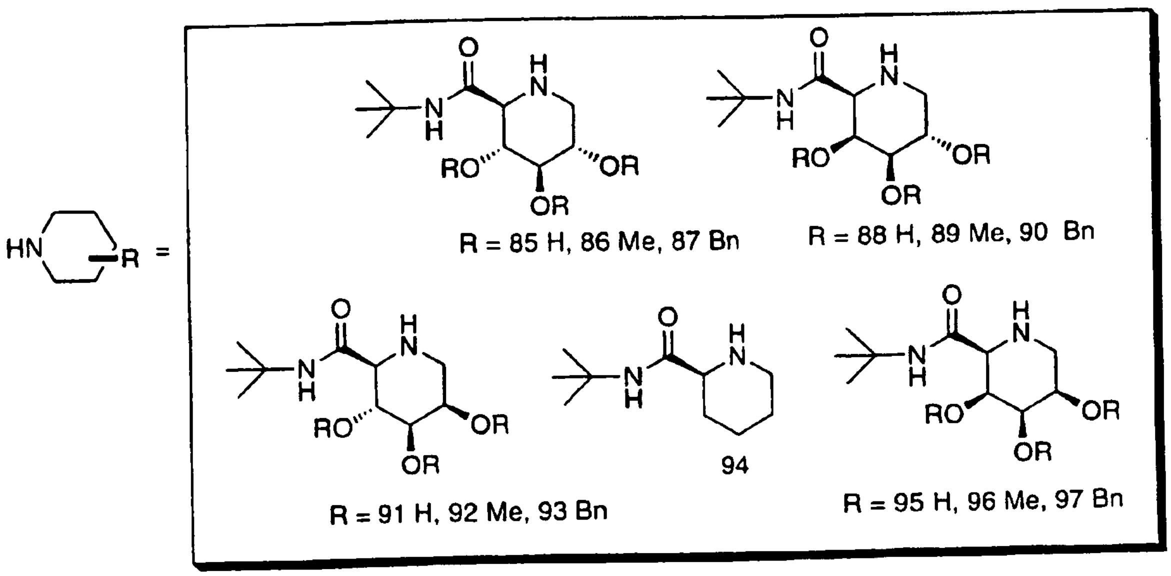
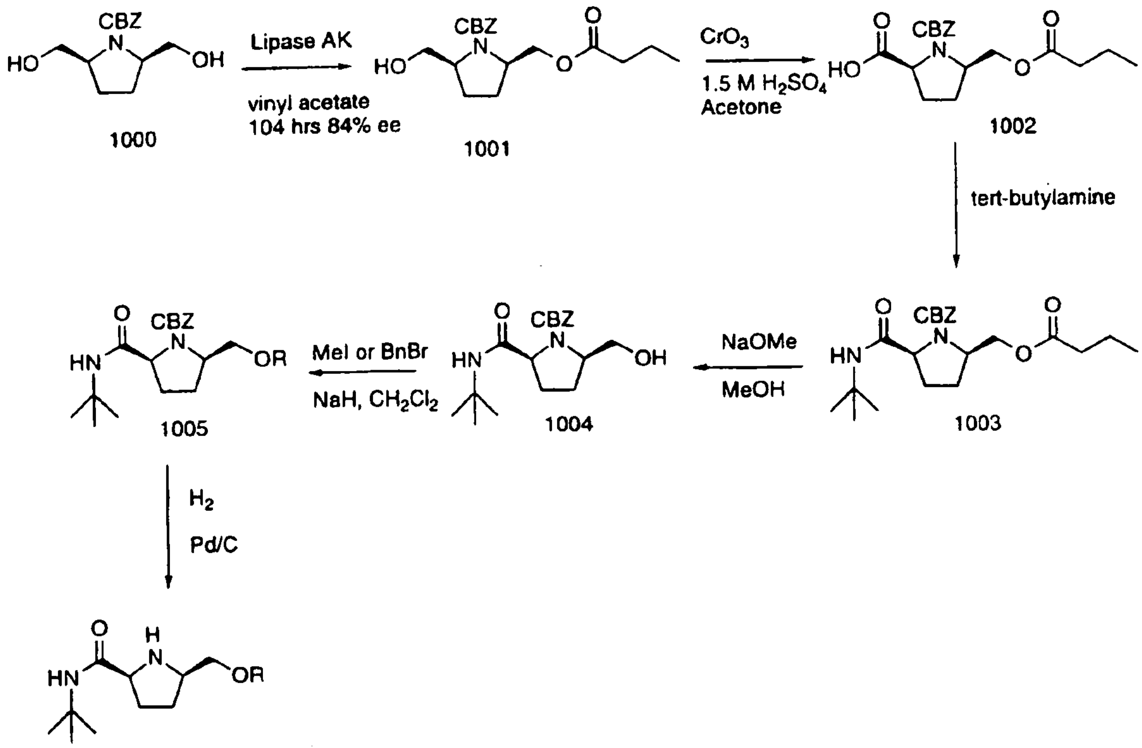
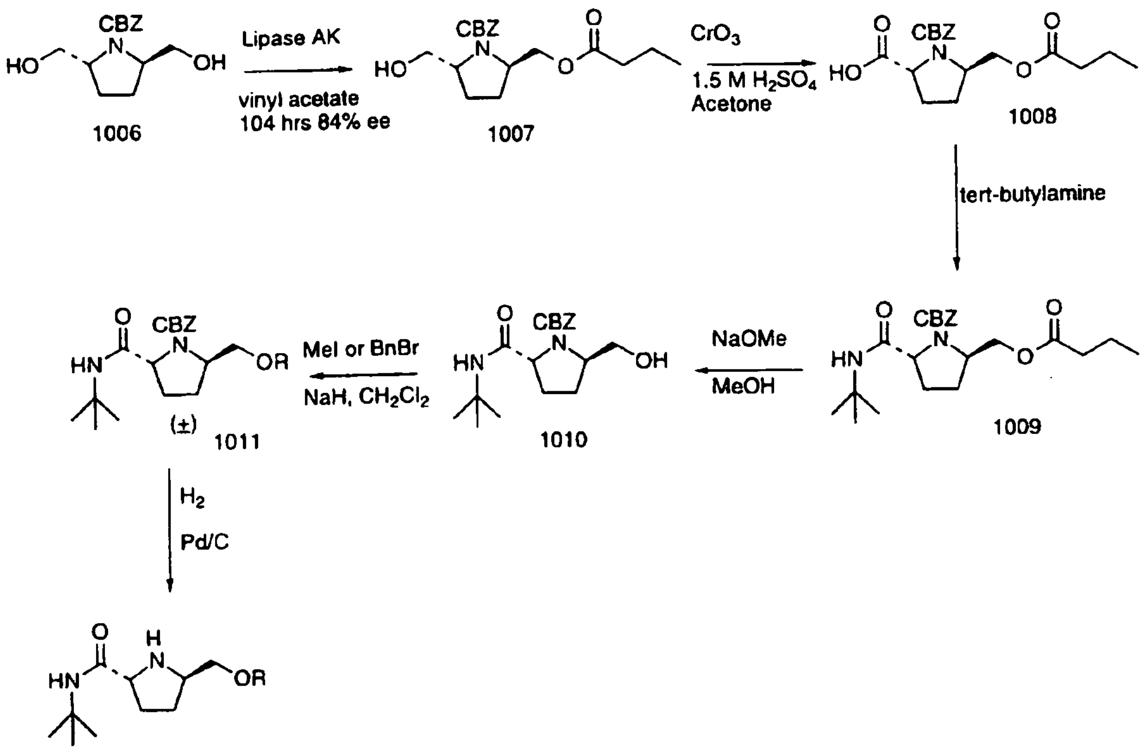

HN
R =
O
N
H
RO
OR
OR
R = 85 H, 86 Me, 87 Bn
R = 88 H, 89 Me, 90 Bn
R = 91 H, 92 Me, 93 Bn
94
R = 95 H, 96 Me, 97 Bn

Figure 14



46:R= H; 47:R=Bn, 48:R=Me

Figure 15



52:R= H; 53:R=Bn, 54:R=Me

Figure 16

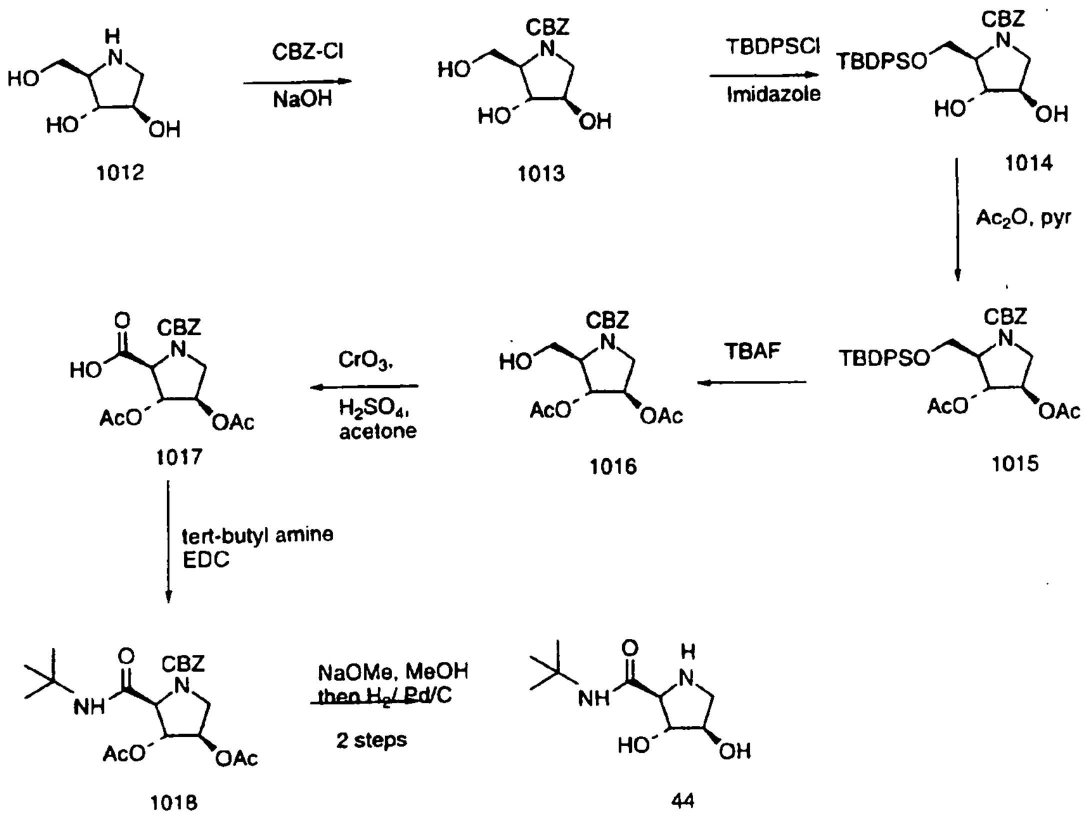
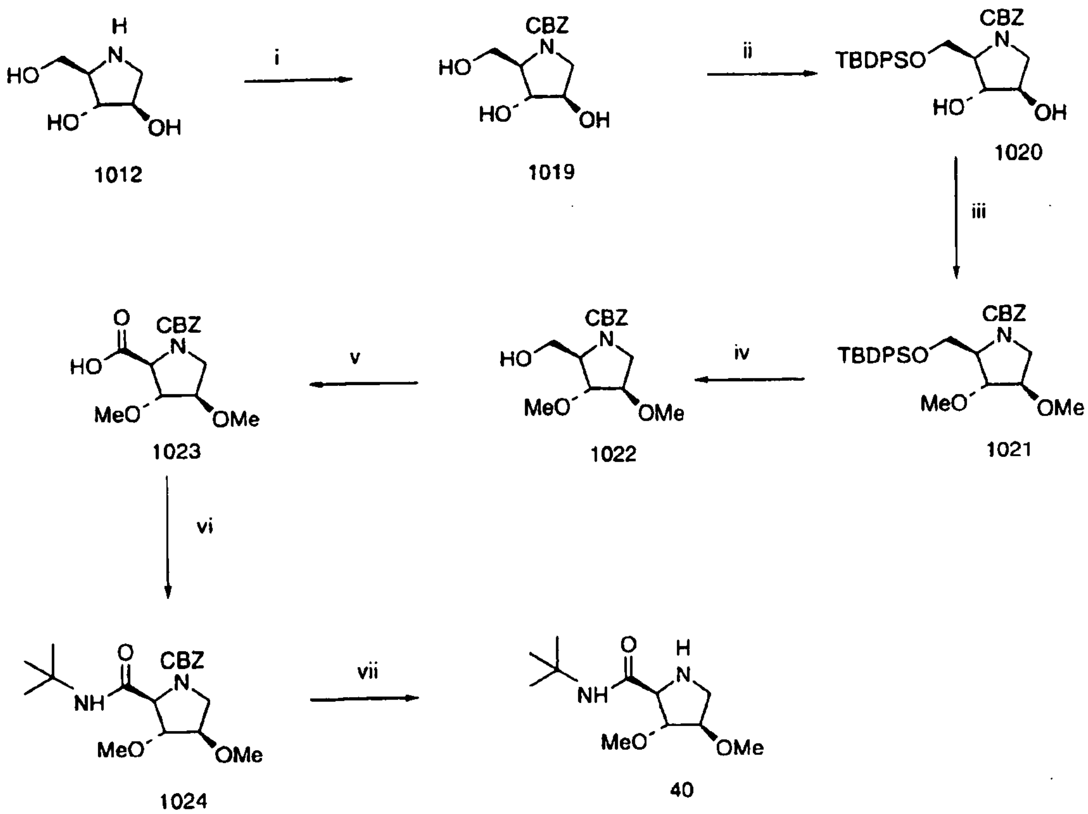

CBZ-Cl
NaOH
TBDPSCl
Imidazole
Ac2O, pyr
TBAF
CrO3,
H2SO4,
acetone
tert-butyl amine
EDC
NaOMe, MeOH
then H2/ Pd/C
2 steps
1012
1013
1014
1015
1016
1017
1018
44

Figure 17



H
HO
HO
OH
1012
i
CBZ
1019
ii
TBDPSO
1020
iii
MeO
OMe
1021
iv
1022
v
O
1023
vi
NH
1024
vii
40

Figure 18

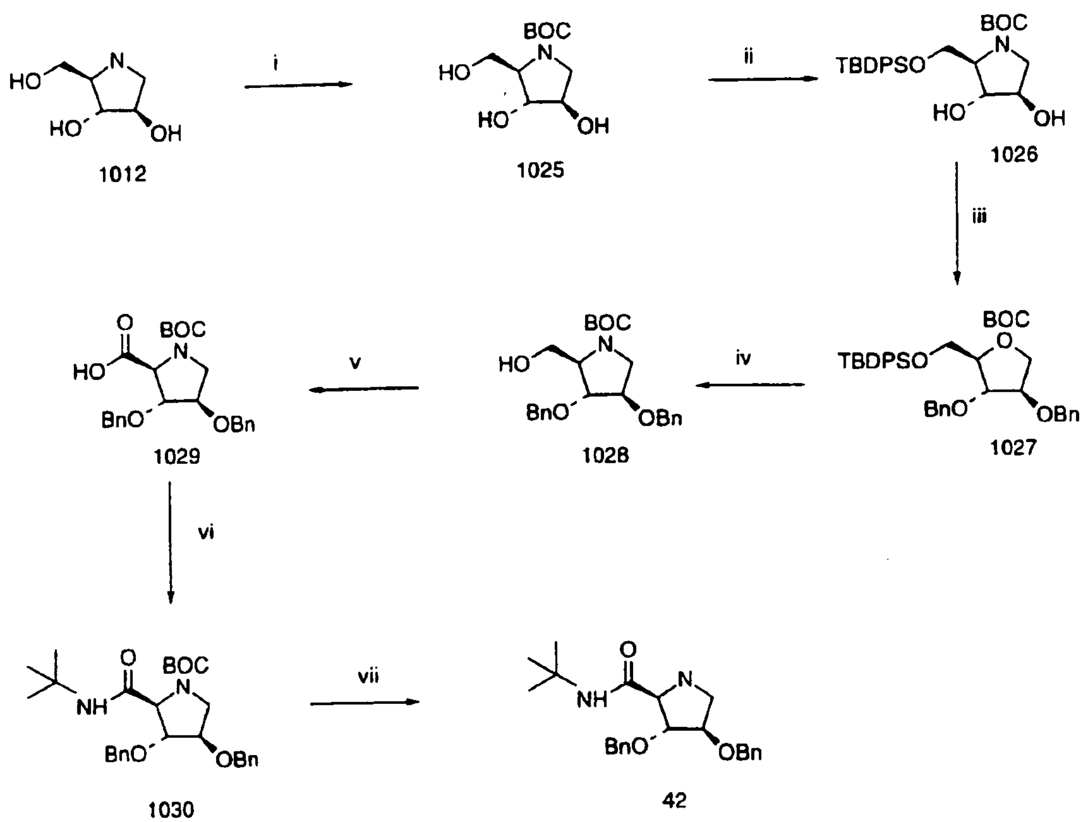
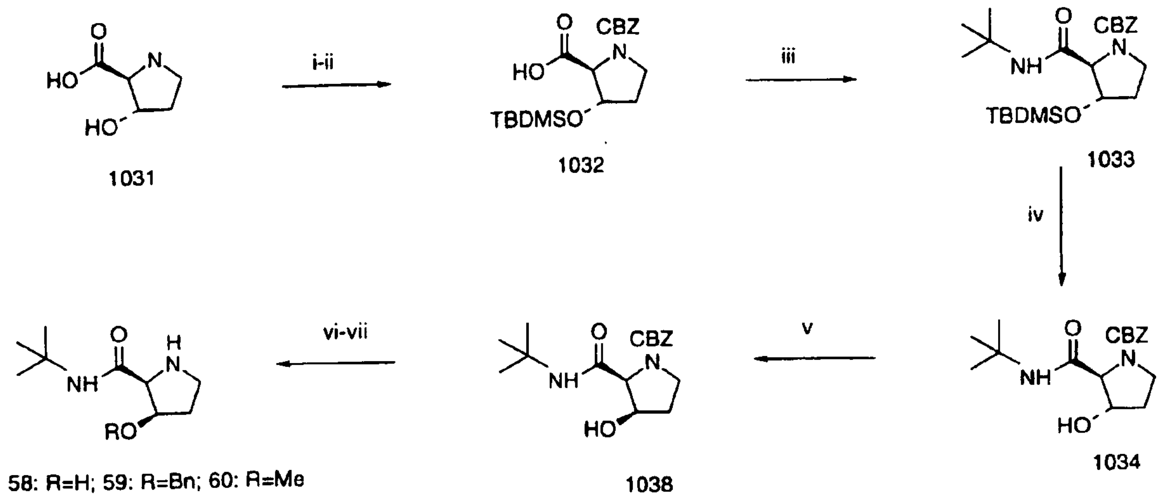

BOC
HO
HO
OH
i
ii
TBDPSO
iii
BnO
OBn
iv
v
vi
vii
NH
O
N
1012
1025
1026
1027
1028
1029
1030
42

Figure 19


1031
i-ii
CBZ
TBDMSO
1032
iii
NH
1033
iv
1034
v
1038
vi-vii
RO
58: R=H; 59: R=Bn; 60: R=Me

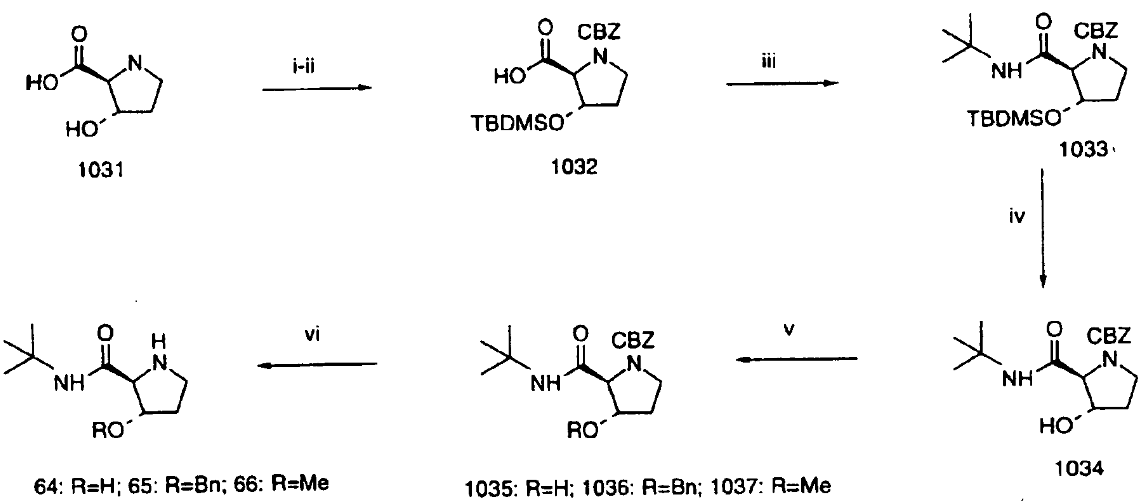

1031
i-ii
CBZ
TBDMSO
1032
iii
NH
1033
iv
1034
v
1035: R=H; 1036: R=Bn; 1037: R=Me
vi
RO
64: R=H; 65: R=Bn; 66: R=Me

Figure 20

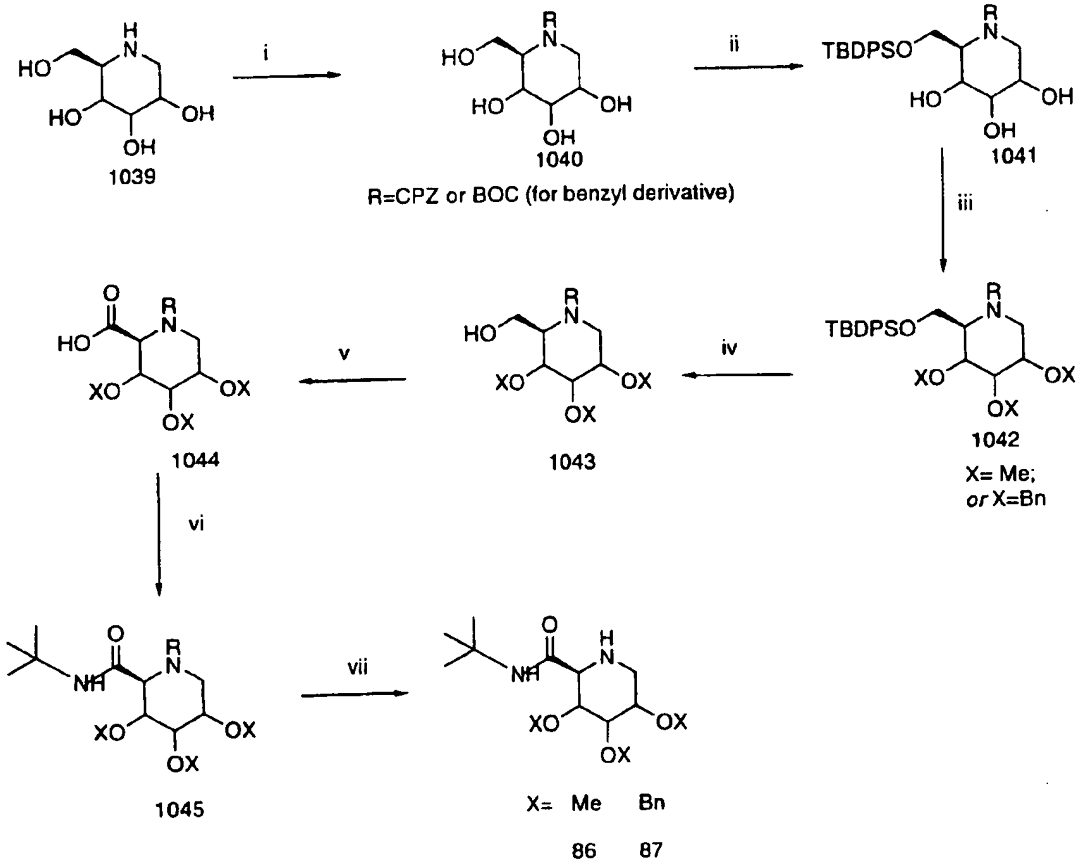

i
ii
iii
iv
v
vi
vii
1039
1040
R=CPZ or BOC (for benzyl derivative)
TBDPSO
1041
1042
X= Me;
or X=Bn
1043
1044
1045
X= Me Bn
86 87

Figure 21

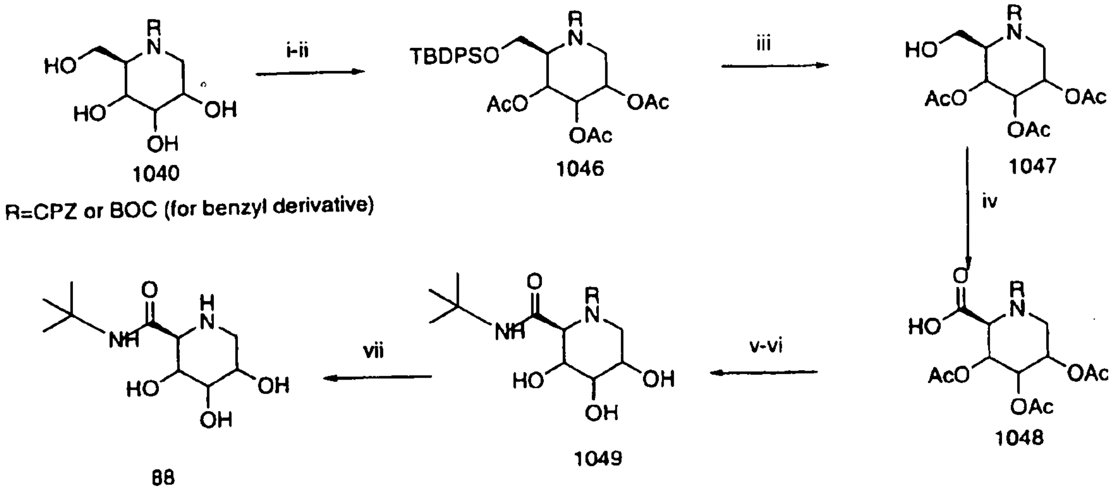
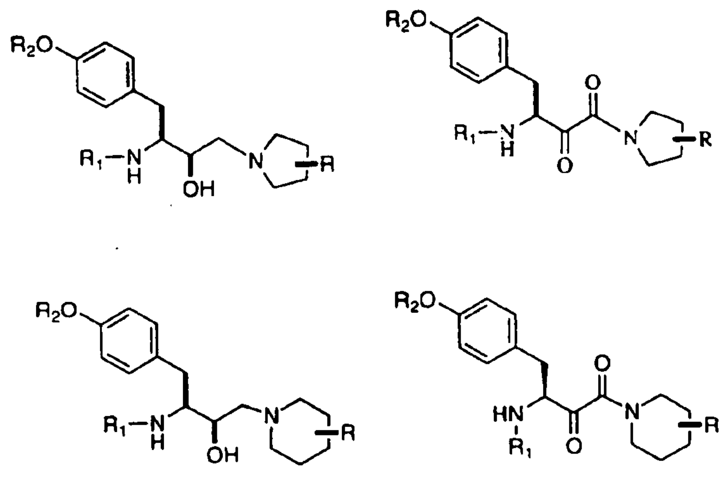
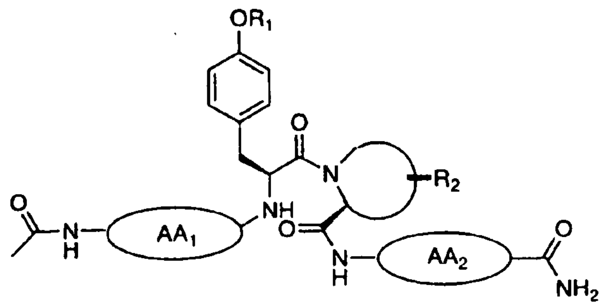

i-ii
TBDPSO
AcO
OAc
OAc
iii
HO
AcO
OAc
OAc
1040
1046
1047
R=CPZ or BOC (for benzyl derivative)
iv
vii
v-vi
1049
1048
88

Figure 22

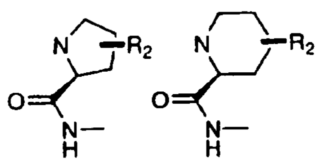


R = various side groups
R_1= CBZ, BOC or other N-protecting group
R_2 = various protecting groups (H, Methyl, Benzyl, p-methoxy benzyl, tertbutyldimethylsilyl, tertbutyldiphenylsilyl etc.)

Figure 23



R_1 = various protecting groups (H, Methyl, Benzyl)



variously substituted pyrrolidines and piperidine analogues

AA_1, AA_2 = natural and unnatural amino acids

Figure 24

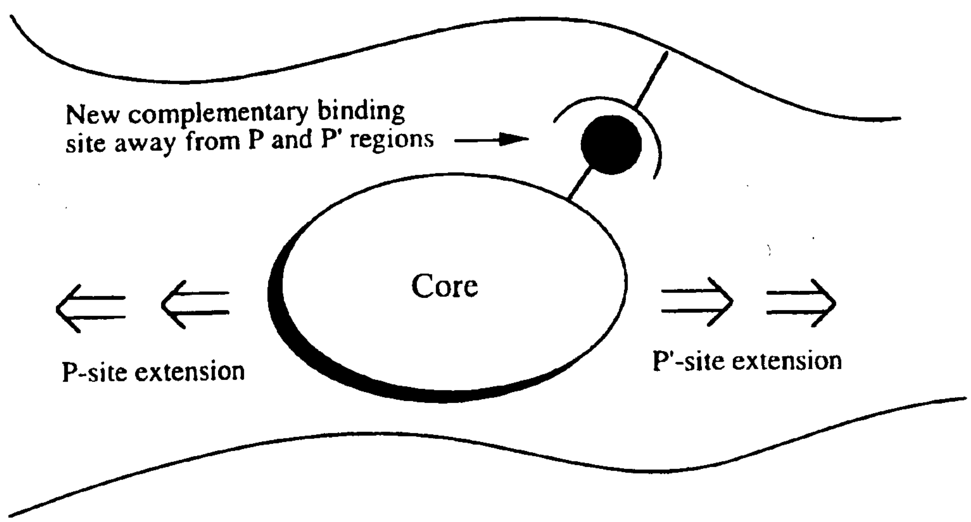
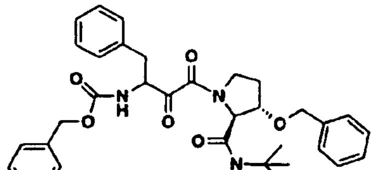
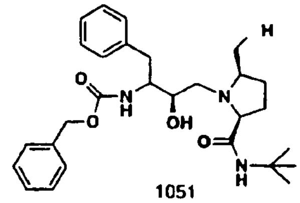

New complementary binding
site away from P and P' regions
Core
P-site extension
P'-site extension

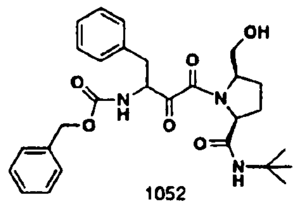
FIGURE 25



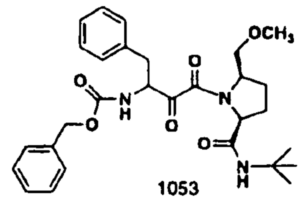
1050



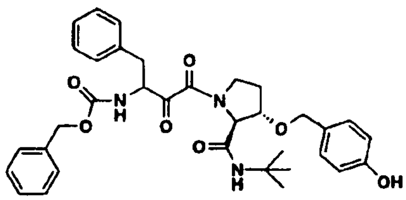
1051



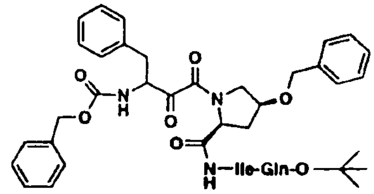
1052



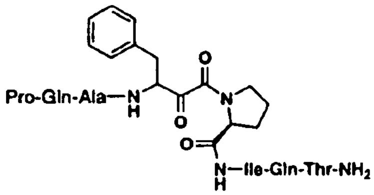
1053



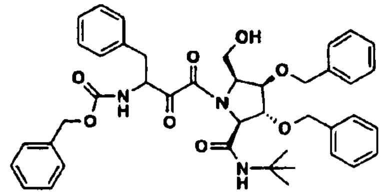
1054



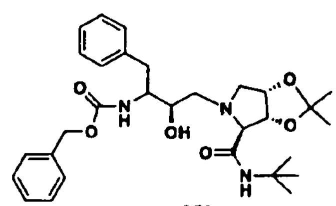
1055



1056



1057



1058

FIGURE 26

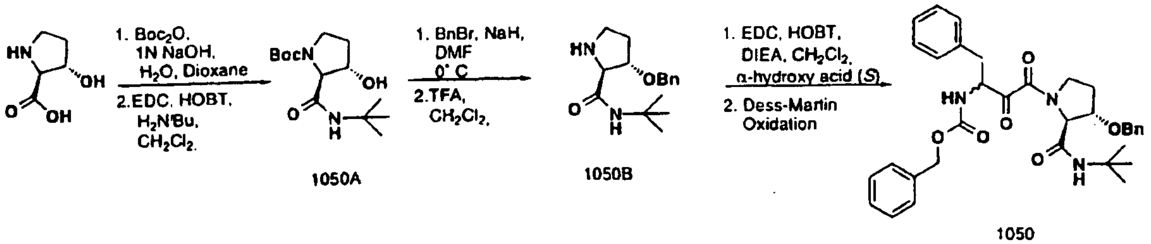

1. Boc_2O, 1N NaOH, H_2O, Dioxane
2. EDC, HOBT, H_2N^tBu, CH_2Cl_2
1050A
1. BnBr, NaH, DMF, 0° C
2. TFA, CH_2Cl_2,
1050B
1. EDC, HOBT, DIEA, CH_2Cl_2, α-hydroxy acid (S)
2. Dess-Martin Oxidation
1050

FIGURE 27

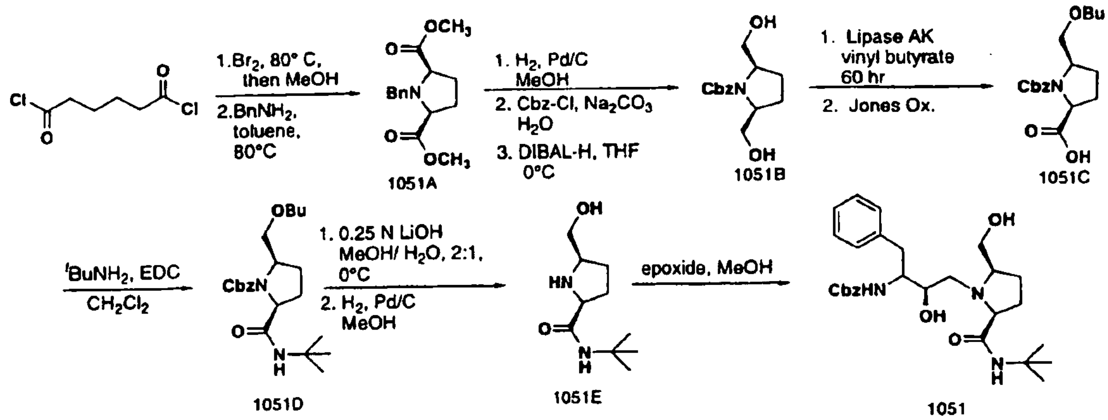

1.Br2, 80° C, then MeOH
2.BnNH2, toluene, 80°C
OCH3
BnN
1051A
1. H2, Pd/C MeOH
2. Cbz-Cl, Na2CO3 H2O
3. DIBAL-H, THF 0°C
OH
CbzN
1051B
1. Lipase AK vinyl butyrate 60 hr
2. Jones Ox.
OBu
1051C
tBuNH2, EDC
CH2Cl2
1051D
1. 0.25 N LiOH MeOH/ H2O, 2:1, 0°C
2. H2, Pd/C MeOH
HN
1051E
epoxide, MeOH
CbzHN
1051

FIGURE 28

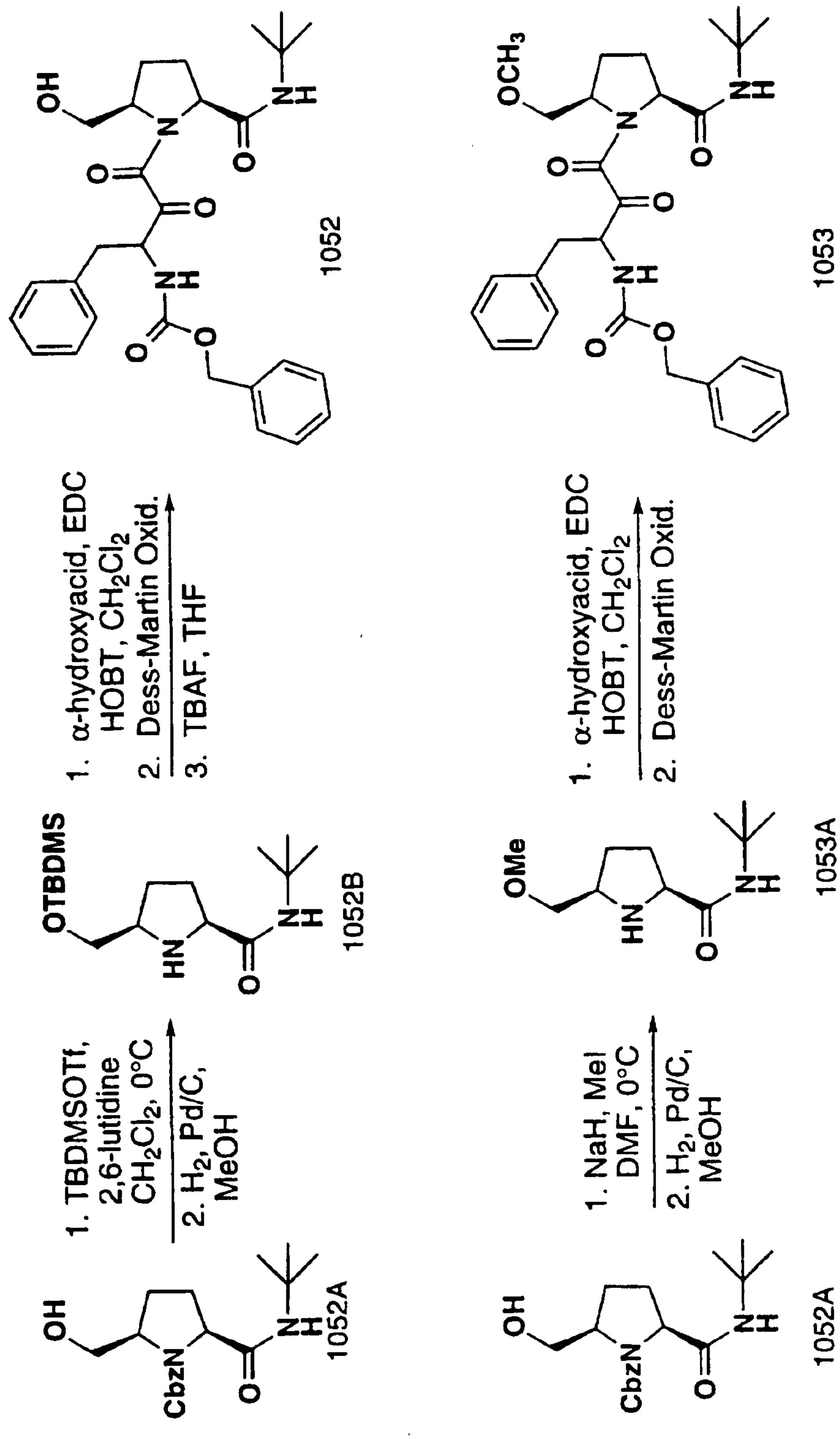
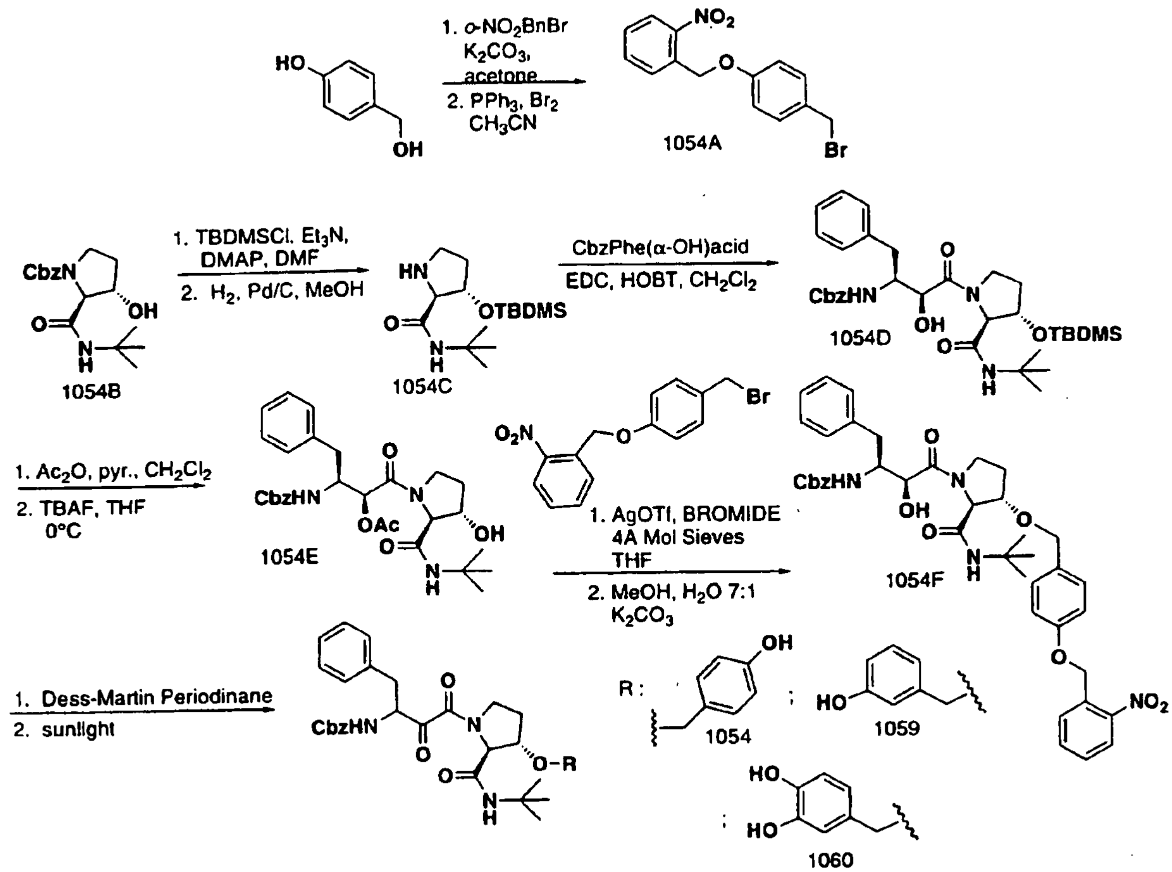
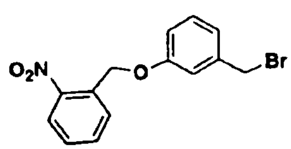
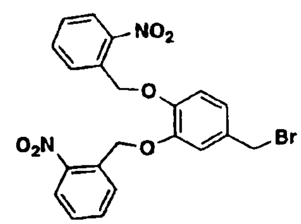

1052A
1. TBDMSOTf, 2,6-lutidine CH_2Cl_2, 0°C
2. H_2, Pd/C, MeOH
1052B
1. α-hydroxyacid, EDC HOBT, CH_2Cl_2
2. Dess-Martin Oxid.
3. TBAF, THF
1052
1052A
1. NaH, MeI DMF, 0°C
2. H_2, Pd/C, MeOH
1053A
1. α-hydroxyacid, EDC HOBT, CH_2Cl_2
2. Dess-Martin Oxid.
1053

FIGURE 29

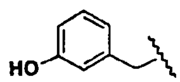


ALTERNATIVES USING THE FOLLOWING BROMIDES:

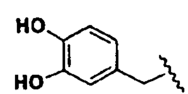




WHEREIN R = WHEREIN R =



1059



1060

FIGURE 30

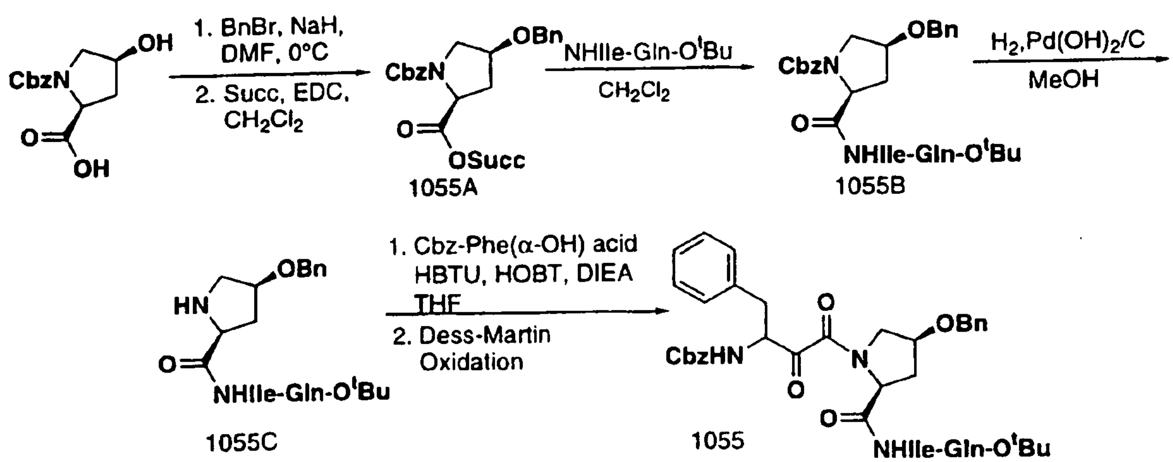
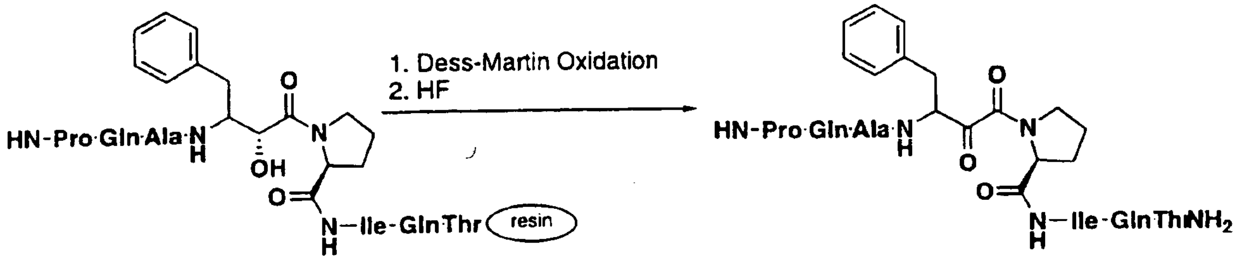

OH
CbzN
O
OH
1. BnBr, NaH, DMF, 0°C
2. Succ, EDC, CH_2Cl_2
OBn
CbzN
O
OSucc
1055A
NHIle-Gln-O^{t}Bu
CH_2Cl_2
OBn
CbzN
O
NHIle-Gln-O^{t}Bu
1055B
H_2,Pd(OH)$_2$/C
MeOH
OBn
HN
O
NHIle-Gln-O^{t}Bu
1055C
1. Cbz-Phe(α-OH) acid HBTU, HOBT, DIEA THF
2. Dess-Martin Oxidation
CbzHN
O
O
N
OBn
O
NHIle-Gln-O^{t}Bu
1055

FIGURE 31



1056A 1056

FIGURE 32

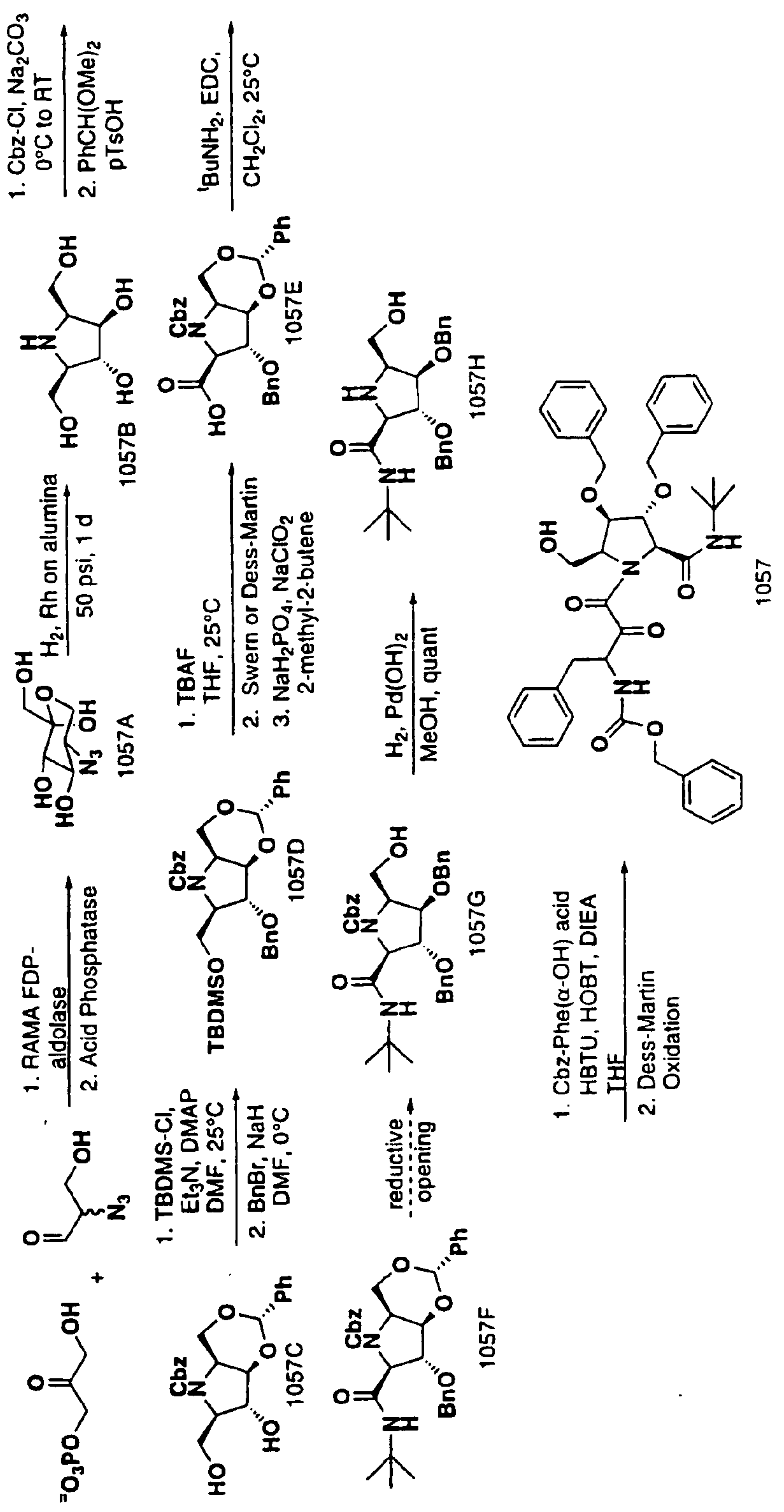

1. RAMA FDP-aldolase
2. Acid Phosphatase
1057A
H_2, Rh on alumina
50 psi, 1 d
1057B
1. Cbz-Cl, Na_2CO_3
0°C to RT
2. $PhCH(OMe)_2$
pTsOH
1057C
1. TBDMS-Cl, Et_3N, DMAP
DMF, 25°C
2. BnBr, NaH
DMF, 0°C
1057D
1. TBAF
THF, 25°C
2. Swern or Dess-Martin
3. NaH_2PO_4, $NaClO_2$
2-methyl-2-butene
1057E
$^tBuNH_2$, EDC,
CH_2Cl_2, 25°C
1057F
reductive
opening
1057G
H_2, $Pd(OH)_2$
MeOH, quant
1057H
1. Cbz-Phe(α-OH) acid
HBTU, HOBT, DIEA
THF
2. Dess-Martin
Oxidation
1057

FIGURE 33

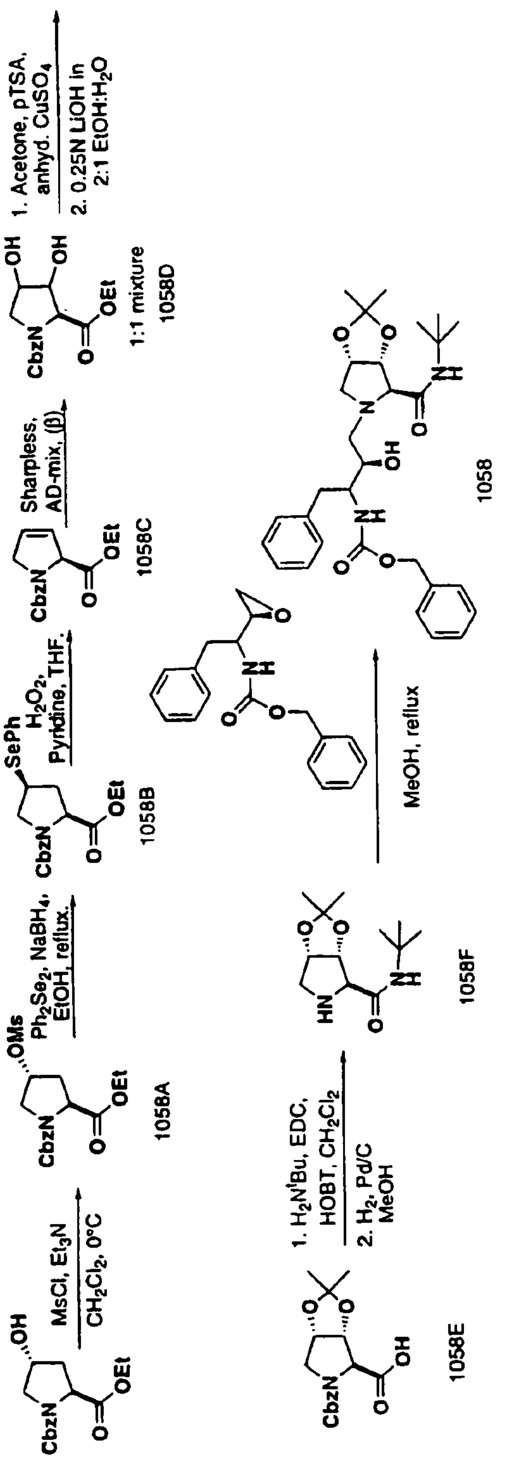

MsCl, Et$_3$N
CH$_2$Cl$_2$, 0°C
1058A
Ph$_2$Se$_2$, NaBH$_4$, EtOH, reflux.
1058B
H$_2$O$_2$, Pyridine, THF.
1058C
Sharpless, AD-mix, (β)
1:1 mixture
1058D
1. Acetone, pTSA, anhyd. CuSO$_4$
2. 0.25N LiOH in 2:1 EtOH:H$_2$O
1058E
1. H$_2$N^{t}Bu, EDC, HOBT, CH$_2$Cl$_2$
2. H$_2$, Pd/C MeOH
1058F
MeOH, reflux
1058

FIGURE 34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19571

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53
US CL : 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, WPI, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,545,640 A (BEAULIEU ET AL.) 13 August 1996 (08/13/96), see especially column 9.	1
Y,P	US 5,532,124 A (BLOCK ET AL.)02 July 1996 (07/02/96), see especially column 4, Summary of the Invention.	1
Y	US 5,171,662 A (SHJARMA) 15 December 1992 (12/15/92), see especially the abstract and column 2, 2nd paragraph.	1
Y	US 5,436,131 A (CONDRA ET AL) 25 July 1995 (07/25/95), see especially the abstract.	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 MARCH 1997	04 APR 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	LYMAN SMITH
Facsimile No. (703) 305-3230	Telephone No. (703) 305-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19571

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19571

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KANETO et al. A Rapid and Simple Screening Method for HIV-1 Protease Inhibitors Using Recombinant Escherichia coli. The Journal of Antibiotics. April 1994, Vol. 47, No. 4, pages 492-495.	1
Y	WANG et al. Synthetic Chemical Diversity: Solid Phase Synthesis of Libraries of C2 Symmetric Inhibitors of HIV Protease Containing Diamino Diol and Diamino Alcohol Cores. Journal of Medicinal Chemistry. 04 August 1995, Vol. 38, No. 16, pages 2995-3002, especially page 2998, first paragraph.	1
Y	THAISRIVONGS et al. Structure-Based Design of Novel HIV Protease Inhibitors: Carboxamide-Containing 4-Hydroxycoumarins and 4-Hydroxy-2-pyrones as Potent Nonpeptidic Inhibitors. Journal of Medicinal Chemistry. 01 September 1995, Vol. 38, No. 18, pages 3624-3637, see especially the abstract.	1
Y	TANABE-TOCHIKURA et al. Anti-human Immunodeficiency Virus (HIV) Agents are also Potent and Selective Inhibitors of Feline Immunodeficiency Virus (FIV)-Induced Cytopathic Effect: Development of a New Method for Screening of anti-FIV Substances in vitro. Antiviral Research. August 1992, Vol. 19, No. 2, pages 161-72, see entire document.	1